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ON THE ORIGIN OF THE FREE CELLS OF SEROUS EXUDATES

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During an investigation of the reactions and characteristics of the lining cells of the serous membranes, I have been able to establish certain definite criteria by which these cells could be identified under all circumstances. These specific criteria were immediately recognized as offering a method by which the relationship of these cells to the free cells of the exudate could be accurately determined, and it is the object of the present report to recount these results. The bearing of these observations upon the physiology of the serous membranes as such, will be presented at another time.

The topographical distribution and morphological characteristics of the free cells of the mammal have been studied with great accuracy and in great detail. In addition, many of the minor details of structure and staining reaction have been considered as specific for cell-types by most investigators, and in this way certain broad classifications in a structural sense have become generally accepted. Obviously the two great questions which arise in regard to these cells are: what genetic relation do they bear to each other and to fixed cells, and to what extent do the differences of morphology indicate differences of physiological capability and functional differentiation?

The arguments between the believers in cell specificity and their opponents have nowhere been more fiercely waged than in regard to the great group of cells which constitute the free cells of the blood, connective tissues and serous cavities. All of the earlier work on this subject as well as much of that of a more recent date has been based upon the classification of cells by slight variations in their form, nuclear structure, and the reactions of their cytoplasmic granules to different stains; and

most of the conclusions drawn have been from a study of fixed material alone. Such studies have led to the discovery of many so-called transition forms, which have been interpreted by many investigators as evidence of the development of cells from one type to another; many of these forms are, however, only individual variations within the same cell-type and do not afford reliable criteria on which to base the formation of cell-series. It is extremely difficult to determine the direction of differentiation of such a series, some authors accepting a certain cell-type as the adult, others interpreting it as the primitive type. In this way fibroblasts have been derived from clasmatocytes and clasmatocytes from fibroblasts, and the small lymphocytes have been considered by some as the young type, by others the senile and final stage. The intervention of a host of experimental methods has succeeded in turning investigation away from the study of cell-series, and has materially assisted in bringing some of the many relationships of free cell-types into clearer perspective, even if such studies have not as yet entirely settled these problems. The work of many investigators with the vital dyes, the study of living cells either in fresh preparations or in tissue cultures, the use of specific depressants or specific stimulants to destroy or increase certain types, and finally the numerous methods of analyzing the cellular activities incident upon and following inflammatory reactions, have gone far toward establishing the specificity of certain cell-types and the interrelation of others. And it has been through some or all of these methods that an introduction has been gained to the study of the physiological activities of the free cells of the mammal.

It is generally agreed by most observers that such cells as the nervous, glandular or muscular elements are incapable of further anaplasia or cataplasia into other permanent cell-types with characteristic and definite new functions. There are many cells however which do not seem to be so completely differentiated, or which may only require a certain stimulus in order to pass into some other type of cell; for example, the endothelial lining cells of the sinuses of the spleen and lymph-nodes are thought to be capable of forming free, living monocytes, cells quite different from the parent stem. May not such cells as the endothelium of splenic sinuses retain undifferentiated characteristics, i.e., not have reached the stage of fully developed endothelium? That the development of these cells has been arrested is all the more possible because it has not yet been established that endothelium in the adult, elsewhere than in certain particular areas, can proliferate and give rise to other

cells than endothelium. Such changes as Harvey (20) has described for the cells of the pylorus are much more likely to represent a final stage in the life history of a cell, hastened in time, and therefore increased in quantity by the stimulus applied, rather than representing a further differentiation of an adult cell-type.

Not the least interesting among the free cells of the mammal are those that are found normally in the serous cavities, and few, those of the circulating blood excepted, have been more intensively studied. These cells have been enumerated and described so often and with so great agreement, that an elaborate description of them is unnecessary here. The majority of these cells are large mononuclear elements, usually free of granules, and capable of locomotion and phagocytic activity. What function they may exercise in the normal state of the serous cavities is as yet entirely unknown, but that they form a most important part of the defensive apparatus in any inflammatory reaction is beyond question. These large mononuclear cells, which are present normally, become greatly increased in number during any irritation. There have been many suggestions advanced as to their origin; they have been derived from the mononuclear cells of the blood-stream, preformed clasmatocytes, fibroblasts, adventitial cells and from the serosal mesothelium. But the principal point of discussion has been as to whether they were derived from the blood-mononuclears or were autochthonous in origin.

On the advent of any foreign body into the serous cavities there is an enormous increase in the cellular content of the exudate, the cell-picture varying with two factors, the nature of the irritant and the time of the observation after its introduction. In so many of the early studies intense reactions were produced by the use of bacteria, turpentine and other violent agents, which induced such tremendous outpourings of polymorphonuclear leucocytes from the blood, and consequent formation of pus in the cavities, that an accurate analysis of the different cell-types met with in the exudate during the course of the inflammation was rendered difficult or impossible. Again many observers used pieces of sterile gauze and other large foreign bodies to produce changes and in this way obtained a typical foreign body reaction involving extensive injury to, and proliferation of, cellular elements in direct contact with the foreign body and a relatively normal condition elsewhere. It was only when suspensions of particulate matter, red blood-cells, killed and practically non-toxic bacteria, and even less irritant materials were introduced, that analysis of the cells of the exudates became possible.

Terminology. In order to avoid misinterpretation it is necessary to take up very briefly the question of the terminology used in this report. *Endothelium* is used to refer to the cells lining blood-vessels, lymph-vessels and the sinuses of spleen and of lymph-glands. The cells that line the pleural, pericardial and peritoneal cavities are designated as *mesothelial* or as *serosal lining cells.* The term *histiocyte,* suggested by Ashoff and Kiyono (1), is used for all of the large, phagocytic mononuclears of the blood and connective tissues, while *monocyte* is used for those of the circulating blood alone, and *clasmatocyte* for those of the serous cavities and the connective tissues. In addition to these, two other terms must be mentioned; they are, *granule* and *vacuole.* No attempt is made to discuss the physico-chemical nature of these intracellular structures, as very little is known about them. In this paper *vacuole* is used to refer to those portions of the cell cytoplasm in which vital dyes are stored, and no attempt is made to study the question as to whether they are preformed or not. On the other hand *granule* is used to refer to those structures which constitute the specific granulation of the blood-cells, and the highly refractive structures which are described as appearing in the irritated serosal cell.

Literature. It has seemed obvious to almost all workers that the erythrocytes and neutrophilic polymorphonuclears, found in the course of an inflammatory reaction of the serous cavities, had wandered in from the blood-stream. But in regard to the great variety of mononuclear cells, there has been no agreement but rather a widespread divergence of opinions concerning their origin, every conceivable source having been suggested and maintained by one or more observers, often with good evidence, often with little or none. In reviewing the literature it was found that while a large number of theories had been advanced they could be analyzed into three general ideas which have been variously combined: *a,* that all the large mononuclear cells of the serous exudates are derived from the similar monunuclears of the blood, and are emigrated blood-cells; *b,* that they are derived either from the cells preëxisting in the cavities or from similar preformed cells of the same type (tissue-mononuclears, clasmatocytes, histiocytes, etc.) and wander in from the neighboring tissues; and *c,* that they are formed by the proliferation and wandering of certain fixed tissue cells, i.e., of fibroblasts, endothelial and lining serosal cells.

Most of the observers who have maintained the hematogenic derivation of the monocytes of the exudate have studied the cells most closely during the early stages of the inflammations. Helly (24) insists that all

the cells of the exudate during the first twenty-four hours, to which period of time his experiments were limited, are exclusively of hematogenic origin. Of course it is essential to remember that Helly considers the adventitial cells of Marchand and the polyblasts of Maximow as parts of the hematopoietic apparatus. He thinks the cells of the exudate are derived from the blood-cells, and those in turn from the more primitive cells of the hematopoietic tissues. Helly admits, however, that other processes may be involved after the first twenty-four hours, but is sure that serosal, endothelial and connective tissue cells have no part in the formation of the exudate during that period. He describes a "swelling up" of serosal lining cells and thinks this is indicative of commencing desquamation, or direct injury, and thinks that once they become loosened from their connections these cells are rapidly destroyed.

The work of von Büngner (2) strongly supports the views of Helly, but he carries this theory even further, and is convinced that tissue-cells cannot at any time produce other than tissue-cells. He introduced gauze sponges into the peritoneal cavity and studied the process of proliferation and encapsulation which took place. The exudate was incidental, and was studied only during early stages. In his study of the processes of proliferation he describes serosal "endothelial" cells as rounding up, dividing and becoming free in the fibrin layers, and considers that they play an important part in the formation of new tissue.

Another group of workers, particularly Schridde and Maximow, have studied the cells occurring in inflammatory reactions in subcutaneous tissues. Their observations are of great importance and assist materially in establishing the nature and genesis of the cells found in serous exudates. Schridde (53), from his studies of the inflammatory reactions in the kidneys from cases of diphtheria and scarlet fever, concludes that the mononuclear cells which infiltrate the tissues are derived entirely from lymphocytes which emigrate from the blood-vessels, and he describes having actually seen the cells in their passage through the vessel-wall. He finds no mitotic figures in the adventitial cells and only very occasionally in the lymphocytes. In general inflammations Schridde (54) believes that there is a continuous emigration of small lymphocytes, a portion of which undergo further differentiation into leucoblasts, which cell-type in Pappenheim's terminology is the progenitor of leucocytes.

Maximow (41), (42), (43), (44) studied inflammation of the connective tissues by inserting small sterile chambers; for example, he used two glass coverslips tied together. By means of this technique he was able

to separate the connective tissue-cells into two distinct varieties, the clasmatocytes and fibroblasts, the former appearing in the glass chambers much earlier than the latter. The clasmatocyte had never been separated sharply from the fibroblast until Maximow's studies. He found that fibroblasts are capable of movement, but that they move much more slowly than clasmatocytes, and that only a violent and long-continued irritation can force them to round up, become phagocytic and to assume any of the characteristics of the true resting-wandering cell. Maximow does not deny the possibility of the complete transition of fibroblasts to clasmatocytes; but in his experimental work he did not find evidence for such a view. The macrophages or clasmatocytes are, according to him, derived from the polyblasts, cells which are everywhere present in the connective tissues, and which are in turn derived from emigrated lymphocytes of the circulating blood. The wandering cells, which are derived from fibroblasts, return to their original type very soon after emigration, send out processes, become flattened, and the nuclei resume their normal appearance. Maximow's (40) embryological conclusions support those obtained from his work on the adult, and are of interest here. During early stages of development he believes in the complete similarity of the lymphocytes, resting-wandering cells and fibroblasts, all being derived from the same mesenchyme cell and capable of transition in any direction. Generally the formation of the wandering cells from fixed elements becomes more and more localized, and is finally limited to the hematopoietic organs which are developed from undifferentiated mesenchyme; until in full grown animals only "the stem of undifferentiated wandering cells, the lymphocytes," retain perpetually the capability of developing in different directions.

The majority of these investigators, as well as others, base much of their argument on specific morphological detail, finding that the cells of the serous exudates have all the characteristics of blood-cells; and it is most interesting to note that many of those most determined in their opposition to the hematogenic derivation of the exudate-cells also use as their principal argument the differences in morphological detail. There is no doubt as to the value of careful study of cellular characteristics and staining reactions, but the many great variations possible within what is admittedly the same group of cells, has made impossible the final settlement of this problem by detailed study of stained material alone. Any attempt to classify the great variety of conclusions reached by those workers who have discarded the emigration hypothesis in favor of an autochthonous origin would be futile. In general they have

emphasized either the part played by increased activity of the preformed free cells of the connective tissues, or the production of new cells by proliferation and desquamation of serosal or fibroblastic elements.

Among the large group of those who support the autochthonous origin of the exudate-cells, Weidenreich and Schott hold a unique place, because they tend to limit the anlage of the exudative cells rather to a single organ (omentum) than to a given type of cell. Weidenreich (60), (61) considers the omentum as wholly analogous to a lymph-gland, the meshwork of the omentum corresponding to the reticulum of the gland, and the free cells and *tâches laiteuses* to the pulp-cells and nodules respectively. All of the cells forming the layers of the omentum are indistinguishable to Weidenreich and he derives the mononuclears of the exudate indiscriminately from them.

Schott (55), working with Weidenreich, has advanced similar conclusions, though given in much greater detail, and from the results of many experiments. His work has been very widely misquoted, in fact no clear comprehension of his views seems to have obtained a place in the literature. Almost everywhere he is assumed to have derived the cells of the exudate from the general serosal cells only. In the normal omentum Schott admits the presence of two types of cells, fibroblasts and lining cells, but considers a separation of these two cells as not always possible since they possess the same morphological characteristics in many instances. In his experiments Schott made a detailed study of the omentum, at various intervals ranging from a few hours to several months, following the injection of erythrocytes and cinnabar. He describes the fibroblasts and serosal cells as forming a cellular group which, during inflammation, show all the properties which characterize "macrophages" while still attached to their cellular connections. They phagocytize small particles of cinnabar and erythrocytes, and occasionally leucocytes and monocytes as well. He concludes that they have the capability of locomotion because they draw in their processes in connection with the omentum, isolate themselves from their neighbors and become free elements. As such they are met with in large numbers in inflammatory exudates. The identity of these phagocytic elements, which have been often described as appearing free in the peritoneal exudate, with the omental cells, he considers proved by the great morphological and functional similarity of the two forms. In his conclusions he says: "If one considers the morphological similarity demonstrated between these transudate macrophages and the omental cells, then one must come to the conclusion that normally the large non-granular cells

with compact nuclei are formed from the elements of the omentum and serosa respectively." Thus it seems that Schott, like Weidenreich, considers the omentum as the principal source of the exudate-cells, rather than any particular group of cells, for he derives them from all types alike.

There have been, however, other workers who have singled out the serosal cells and considered them the fundamental and perhaps the sole anlage of the free cells of the exudate. Heinz (21),(22), (23) produced inflammations in the serous cavities by injections of iodine, turpentine, and bacterial emulsions. After an inflammation induced in this manner he observed a vigorous regeneration of the serosal mesothelium, the new-formed cells having the typical appearance and behavior of young embryonic cells. The mononuclear cells which he found in the exudate he considers as being "undoubtedly endothelial cells from the serosa." He describes them as large, flat cells with large, vesicular nuclei usually having two nucleoli and true nuclear membranes. In the regenerating serosal cells he describes granules which move about continuously. These granules Heinz emphasizes as very highly refractive and numerous, but he does not discuss their staining reactions. He concludes that the cells of the pleural lining are capable of amoeboid movements.

Many unique efforts have been made in an attempt to settle the various questions involved in the origin of the cells of the exudate, but none have been more interesting and ingenious than those dealing with thorium-X poisoning. Lippman and Plesch (28), (29), by giving exactly graded doses of this radio-active substance, have been able to destroy all of the circulating leucocytes. It is well known that thorium-X affects primarily the bone-marrow and lymph-glands.[1] After aleucocytosis of the blood-stream had been established, exudates of the serous cavities were induced by the introduction of erysipelas cultures and then studied. Using swine erysipelas in the pleural cavity of normal control animals they obtained a thick exudate consisting chiefly of polymorphonuclear leucocytes. When however the animals had been exposed to thorium-X and then injected with these cultures, a pleural exudate was obtained consisting solely of mononuclear cells. They describe these mononuclear cells as showing every possible stage of transition from pleural mesothelium to cells which resemble small lymphocytes. The pleural lining cells round up, become loosened from their connections,

[1] For discussion of the general results of thorium-X poisoning see Pappenheim and Plesch (47), (48), Mass and Plesch (31), Hannemann (19) and Plesch, Karczog and Kutman (49).

and in this way give rise to large mononuclar, non-granular cells which, because of their phagocytosis of red blood-cells, they consider as macrophages; and these cells divide producing smaller cells which have the same characteristics. In order to eliminate the possibility of the derivation of exudative mononuclear cells from adventitial and perivascular cells, they introduced aleuronat intermuscularly. When normal animals received this treatment there was a rich exudate of mixed cells, but in an aleucocytized animal this injection produced no exudate, only gangrene and necrosis; and they therefore conclude that adventitial cells and perivascular cells can be excluded as a source for the mononuclear cells of the exudate in their experiments. When irritants were introduced into the peritoneal cavity of an aleucocytic animal they found a somewhat different reaction from that of the pleura, and therefore think that these two show "a different biological reaction." This peculiar variation consists in the presence of numerous polymorphonuclear leucocytes, which are indistinguishable from those of the blood, and which appear along with many mononuclears similar to those already described for the pleura and are derived by these authors from the serosal cells. They find no transition forms, all the polymorphonuclear leucocytes appearing in the exudate as fully developed cells. Lippman and Plesch suggest that there may be undifferentiated cells, similar to the hematoblasts and the leucoblasts of the bone marrow, which are retained in the omentum and which can give rise to leucocytes. They also suggest that these leucocytes, found in the peritoneal exudate, may be derived from a physiological reserve depot which is resistant to thorium-X.

Marchand (36), (37), (38), (39) on the other hand selects the adventitial cell as the most important progenitor of the free exudate-cells. His description of the living omentum during an inflammation caused by a sterile foreign body is the most accurate in the literature. He finds that the serosal cells swell up after irritation, become filled with fine vacuoles and put out processes, thus changing from their normal pavement-like arrangement to an anastomotic one somewhat similar to that of fibroblasts. On the other hand, the adventitial cells, which are normally present in great numbers, swell up as the result of this irritation and are finally attached to the vessel wall only by the finest of peduncles. The nuclei of these cells are large, oval or round, with large chromatin granules. The presence shortly afterward of similar elements in the exudate, large numbers of which are phagocytic, Marchand considers as proof of the origin of these cells from the adventitial cells. In

general he differentiates the lining cells from the macrophages arising from adventitial cells, by the largeness of their nuclei and by the presence of nucleoli.

Of all the work done on the peritoneal exudate that of Pappenheim and his school is perhaps the most painstaking, and his analysis of the work done in this field stands entirely alone. It is true that Pappenheim has in many ways added unnecessary and even confusing complexity to the classification of cell-types and cell-genealogies, but nowhere else is there in any branch of hematology such accuracy of observation and such clarity of analysis. Pappenheim has studied the serosal exudates with three general methods, *a*, by producing the exudate in otherwise normal animals, and studying the cells with all the great variety of hematological technique for which his school is so well known; *b*, by irritating the serosal membranes in animals, which had been previously vitally stained; and *c*, by combining one or both of these methods with the Lippman-Plesch thorium-X treatment. Pappenheim agrees with Lippman and Plesch that the thorium experiments indicate that the lymphoid cells of the serosal exudates cannot be derived from the cells of the circulating blood. He suggests that the stem cells of primitive type may be more resistant to thorium than the more highly developed myelocytes and other intermediate cells of bone-marrow and blood. In this way the stem-cells of the hematopoietic tissues, including general connective tissue, from which he derives the exudate-cells, remain intact. Pappenheim indicates that the serosal lining cells, lymph and blood vascular endothelium and the polyblastic clasmatocytes are possible sources from which the exudate-cells may be derived. The participation of the serosal lining cells he rules out for several reasons. In the first place he does not believe that the cells lining the pericardial cavity and subarachnoidal spaces are true serosal lining cells comparable to those of the peritoneal and pleural cavities, but finds that in a comparative study of inflammatory reactions in these four localities exactly similar exudative cells are present in all. Secondly, in animals which have been vitally stained before the inflammatory reaction was produced, the exudate is always found to contain vitally stained cells, while the serosal cells show no vital stain. And finally, he describes the vitally stained cells of the omentum as being very numerous and as making up the largest part of its cellular constituents, but he finds them clearly differentiated from the serosal cells and there are no transition forms between them. True serosal cells are present in the exudate, but these in no case show the same reaction as the usual phagocytic mononuclear

cells. Pappenheim feels that the endothelium of blood-vessels can not be excluded as a possible source in the formation of histiocytes, but finds no positive evidence in his experiments on this point. Thus only the polyblastic clasmatocytes and adventitial cells of the omentum and subserosal tissues remain as possible sources for the exudate-cells, and it is to these that Pappenheim refers their origin. This conclusion is based upon certain morphological and physiological similarities in these two groups of cells. Both the free cells of the omentum and the large cells of the exudate have the same tinctorial reaction in fixed preparations, similar nuclear conformation, comparable type of amoeboid movement, identical reaction to vital stains, and similar phagocytic capabilities.

In conclusion Pappenheim and Fukushi (45) say: "(1) The exudate-cells of the serous cavities are not of a hematogenic nature, and are not to be considered as true small lymphocytes nor true large monocytes, but as locally formed, histiogenic, lymphoid cells. (2) They are not derivatives of the lining cells; these lining cells vary in nature in different cavities and are not vitally stainable. (3) The cells of the peritoneal exudate are very probably derivatives of the Marchand adventitial cells and the ubiquitous wandering mesenchyme cells of the subserous connective tissues. (4) The large cells are first formed and later the small inflammatory cells, the small originating from the large by continuous proliferation."

Szecsi (56), and Szecsi and Ewald (57) believe that endothelium of the vessel-walls, perithelium and serosal mesothelium constitute an ubiquitous tissue of mesenchymal origin which is capable at all times, under the influence of certain irritants, of functioning hematopoietically. They believe further that leucoblasts originating thus in loco are capable of being transformed into cells having special granules. They describe large myelocytes arising in this manner and developing further into adult granular "special cells", as had already been described for eosinophiles and mast cells (Pappenheim, Weidenreich). This unusual development of leucocytes, they think, yields cells which are larger than those of the blood-stream and which have plump nuclei and well developed granules. They consider the various forms under which the mobilized and active mesothelial cells are to be met with as having collectively a genetic connection. By the growth and rounding up of protoplasm of the mesothelial cells they derive first, the lymphoidcytes and macrophages. From these in turn are derived, by "nuclear indentation," monocytoid forms with leucoblastic nuclei. From these large

lymphoid cells, by proliferation of the monocyte forms or by their "becoming granulated", are derived large cells with special granules, and small eosinophilic myelocytes and leucocytes.

METHODS AND MATERIAL. Two general methods were used for the experiments upon which this report is based. Animals were stained by the intravenous administration of vital dyes, an irritation of one or more serous cavities induced, and the exudative and tissue-cells studied in living and fixed preparations. In other animals the staining and irritation were accomplished by introducing the solution of the dye directly into the serous cavity, thus giving the cells of the exudate and those lining the cavity every opportunity to ingest the dye. In all cases the cells of the exudate, the omentum, and cells scraped from the serous surfaces were studied living, both with and without supravital staining.

The dyes used for vital staining were Grubler's trypan blue, Niagara blue 3B, and crude Niagara blue, usually in 1 per cent aqueous solution but sometimes in the same strength in physiological saline. The crude Niagara blue was found to be an admirable agent to induce a mild irritation resulting in a moderate cellular exudate, and was used in several experiments. The erythrocyte-suspensions were prepared in the usual manner by allowing the blood to flow into a 2 per cent solution of sodium citrate and subsequently washing the corpuscles in physiological saline. This technique was varied by defibrinating the blood in physiological saline and not washing the cells. In some experiments whole blood was given, in some laked erythrocytes. The results were quite similar, except that laked blood produced a somewhat more rapid change in the cells. The injections were usually given at 48-hour intervals, this procedure was varied in special cases; and the usual technique for intravenously stainnig a rabbit was six to ten doses of 10 cc., given at intervals of 24 and 48 hours. The animals used were cats and rabbits.

The operation for removal of the omentum consisted in the usual high left rectus incision; the omentum was divided at the head of the spleen and the right half first removed, then that part attached to the spleen and stomach was removed, great care being taken not to injure the spleen and pancreas. The animals had uneventful recoveries and several weeks later were used for experiments involving vital staining and the usual intraperitoneal irritation.

The majority of the experiments were carried out on the peritoneum but all crucial ones were repeated on the pleura, some of the experiments upon both pleural and peritoneal cavities were carried out in the same animal.

EXPERIMENTAL RESULTS

It is essential, before presenting the details of the experimental work, to indicate briefly the cycle of events which take place in the formation of serous exudates as revealed by these experiments. None of these conclusions are entirely new, each in part being represented in the ideas and findings of some of the many previous observers, but it is hoped that the present analysis will assist in clarifying the extremely complicated state of the subject.

When a sterile peritonitis is induced with any agent, e.g., a suspension of foreign erythrocytes, there is an emigration of cells from the blood-stream consisting at first of polymorphonuclear leucocytes but these are rapidly followed by monocytes and to a less extent by lymphocytes. Many of the monocytes and lymphocytes and to some extent the polymorphonuclear leucocytes are undoubtedly already extravascular. After about 24 hours, the smaller preformed clasmatocytes of the omentum in particular, and of the subserous tissues in general, reach the cavity, followed more slowly by the larger members of this series which appear between 30 and 40 hours after the beginning of the inflammation. From this time on new-formed clasmatocytes arising especially from the proliferation of those situated along the blood-vessels, the adventitial cells of Marchand, continue to be poured out into the peritoneal cavity. As is natural these cells are of every possible size because of their rapid proliferation and tendency to clump and fuse, so that every stage of cell from enormous, multinucleated giant-cells to very small monocytes are to be found in any specimen of peritoneal exudate. During all of this time, and later, there continues to be some emigration of blood-monocytes into the exudate, and in the case of repeated injections, of a small percentage of polymorphonuclear leucocytes as well. Beginning early there are always a few desquamated serosal lining cells in the exudate, at first they have the typical flat appearance, but change in a very specific manner as the course of the irritation progresses. This change is probably an abnormal, because modified and exaggerated, increase of the functions normally present in these cells; but certainly this change is never a further differentiation toward the clasmatocytic type. They round up in situ, proliferate slowly by mitotic division and possibly also by amitosis (this point is still unsettled) and occasionally desquamate. The cells that have become loosened and finally desquamate, are not free living clasmatocytes but correspond to the cells in situ, and while they may either degenerate immediately, or

continue to live for a short time, they do not assume the indiscriminate phagocytic activity of clasmatocytes, nor do they proliferate to form new cells of this series. The longer the irritation lasts the more the cells present in the exudate tend to assume a uniform type, that of relatively small monocytes. The number of free serosal lining cells varies with the intensity of the continued irritation; at times large groups will desquamate together and these usually show evidences of degeneration and presage the formation of adhesions, but if the irritation remains at a low level, then single cells round up, fall off and slowly disintegrate. They are capable of some phagocytic activity, but never in such high degree as is evidenced by the monocytes. It seems assured that they represent a very highly specialized cell, acting as a lining cell for the serous cavities but capable of active secretory activity.

This work has given no evidence of the derivation of cells containing special granules, neutrophiles, basophiles or eosinophiles, from the serosal lining cells, or from the hematogenic and histiogenic monocytes; the latter contain many granules, but not of a comparable character, and there is no indication that they become differentiated to the special granular type.

Evidence concerning emigration. The majority of the earlier investigations have supported the theory that the cells of the inflammatory exudates are derived from the blood-stream by emigration. But the more recent workers have in general argued in favor of an autochthonous origin for these cells. The discussion which has thus arisen has not so far resulted in any theory which is adequate to explain the variety of experimental findings.

Lippman and Plesch, who have vigorously maintained the autochthonous origin of exudate-cells have perhaps furnished the best evidence in favor of this theory, and have done more toward excluding emigration than any other observers. The special granulated leucocytes found by them in the peritoneal exudate of aleucocytized animals could not have been derived from the free cells of the circulating blood and therefore some other source must be postulated for these cells. Three sources have been suggested as possible—the serosal mesothelium, some stem-cell located in the tissues, and blood-cells which had wandered out into the tissues and which were therefore somewhat protected from thorium-X.

It would be interesting to know if the polymorphonuclear leucocytes found by Lippman and Plesch in the exudates produced experimentally in the peritoneal cavity were eosinophiles, because all observers except

Szecsi agree that the neutrophilic leucocytes are derived from the blood, while there is no agreement with regard to the eosinophiles. In my own experiments the number of eosinophiles in the early stages was very small but increased rapidly as the inflammatory reaction became chronic, and later often the entire group of polymorphonuclear leucocytes was eosinophilic. In the rabbit this was more marked than in the cat, where some neutrophiles and an occasional basophile were always intermixed with the preponderating eosinophiles. In rabbits injected with washed erythrocytes there were in many instances no neutrophiles present after four injections. The eosinophiles of the exudate usually have nuclei which are quite similar in appearance to those of the cells of the blood-stream, but the granules are consistently smaller and somewhat less regular in size. They are produced by any chronic inflammation but seem to be more pronounced in those animals where the irritant employed was washed or laked erythrocytes. That there is a definite relationship between the eosinophiles of the exudate and the injected erythrocytes can not be positively concluded until quantitative studies have been made. There are always a few red blood-cells present during inflammatory reactions of the peritoneal cavity and in those experiments, where other agents than erythrocyte-suspensions were used, the red blood-cells usually present may be sufficient to cause the emigration or formation of the eosinophiles, which appear more slowly and in smaller number in these experiments.

The effects of thorium-X are still somewhat poorly defined, and while it is obvious that the circulating blood becomes entirely aleucocytic, this phenomenon may not be associated with entire destruction of all true blood-cells. It is a well-known fact that large numbers of eosinophilic myelocytes are present in lymph glands, so that the bone-marrow cannot be considered as the sole region in which development of granular leucocytes can take place. Whether there is any primitive stem-cell such as Pappenheim postulates in the general connective tissues, or only in such areas as the *tâches laiteuses* of the omentum, lymph glands, etc., remains to be determined. The fact that the pleural cavity does not give the same reaction as the peritoneal has been pointed out by Lippman and Plesch, and may indicate that the cells in question are derived from the omentum or that these emigrated cells are to be found more abundantly in such tissues as border the peritoneal cavity. The evidence which I have on the question of mesothelial participation in the formation of exudate-cells seems to eliminate them entirely from consideration and thus leaves only two possibilities for the origin of these cells. Either there is a stem-

cell in the general connective tissues as well as in bone-marrow which is capable of giving rise to leucocytes having all the characteristics of the blood-polymorphonuclears or else the previous emigration and storing of cells in the tissues must be postulated.

Lippman and Plesch, on the other hand, have undoubtedly established that some of the mononuclear cells of the exudate are derived autochthonously but they have not succeeded in establishing that in normal animals the blood-cells may not be concerned as well, and by emigration form some part of the serosal exudates. This could only be established by some particular point of differentiation. The much smaller number of Kurloff corpuscles found in the monocytes of the exudate, as compared with those of the blood-stream, has been used by Pappenheim, Szesci, *et al* as a differential point, but this does not disprove the presence of at least a small number of blood-monocytes in the exudate, and in addition there is no evidence against the possibility that these Kurloff corpuscles may not rapidly disappear after emigration or be confused with phagocytized material taken up by the cells after they have entered the serous cavities.

The question of the participation of blood-cells in the formation of exudates has been somewhat clarified by two of my experiments which were in some degree accidental. Among a group of rabbits which were being vitally stained by large doses of trypan blue at 24-hour intervals, two developed intravascular staining of practically the entire leucocytic series, especially the polymorphonuclear leucocytes and the transitionals. Not all of the cells were stained but in any blood-smear about 75 or 80 per cent were. In these animals peritoneal irritation was induced, and the animals sacrificed at 12 and 20 hours respectively; in both cases practically all the granular polymorphonuclears of the exudate were stained; in the first about 60 per cent of the mononuclears, and in the second somewhat less than 40 per cent were stained. It is very difficult to explain the reason for the staining of these leucocytes in some animals and not in others. So far this general staining only occurred in two experiments, although an occasional stained polymorphonuclear can be seen in the tissues in many animals. The subject of the vital staining of the blood-cells has been reviewed by Downey (10), (11) who was able to stain the entire series of white blood-cells by ligating a vein in two places and injecting the dye into this area. Under these conditions the cells presumably have equal opportunities with the tissue-cells for ingesting the dyes and it seems probable that the reason for the usual lack of staining of the elements in the blood-stream is a lack of opportunity on the part of the cells to come in contact with the dyes for

a sufficiently long time. The great rapidity with which foreign material leaves the blood-stream has been shown by the recent work of Drinker and Shaw (13) which is the most exact and accurate of a long series of studies on the general subject of the fate of particulate matter in the organism. On the other hand, Evans and others regard the process of vital staining as specific for the histiogenic cells, and suggest that any staining of blood-cells found in the tissues is due to injury of the cells. In my experiments there was no evidence of cellular injury and this finding tends to support Downey's view, but it must be considered as still open to question, because the same procedure has not in every case produced the complete staining of the blood-cells and the possibility of the staining being dependent upon some injury has not been ruled out. The evidence, while not conclusive as regards the question of the capability of the blood-cells to absorb and store vital stains, can certainly have no other interpretation than that a large part of the cells appearing in the serous exudates during the first few hours of an inflammation have wandered out of the blood-stream. That the polymorphonuclear leucocytes are derived in great masses from the blood-stream is, I think, absolutely certain, and in all probability all of them come either from the circulating blood or from cells which have temporarily emigrated into the tissues. Some of the eosinophiles probably are derived from the eosinophilic cells of the *táches laiteuses*. With regard to the mononuclears there is evidence that a part of them is derived locally, while the experiments described above indicate in turn that part is derived from the blood-stream. The question naturally arises regarding the possibility of the cells present normally in the serous cavities proliferating rapidly and giving rise to a part of the cells of the exudate. This question has been examined by several experiments, in which the serous cavities of animals were opened and all the free cells washed out by lavage with warm physiological saline. The animals were then stained by intravenous or subcutaneous injections and the subsequent reaction of the exudate studied. In no case was any difference noted; this similarity in experimental results does not exclude the possible participation of these cells in forming the exudate, but does indicate that the same process takes place in their absence.

The evidence already presented has been supported by that derived from another group of experiments, instigated in an effort to settle the question of the participation of the serosal lining cells in the formation c free cells. In these experiments animals were stained vitally by intravenous injections of trypan blue until a maximum degree of acute

staining was obtained; intraperitoneal injections of washed red blood-cells were then given and the exudate examined at intervals. In the early stages of the inflammation there were a very few monocytes which contained vital dye. These same vitally stained cells were also found in normal exudates, and they showed all the staining characteristics of free clasmatocytes. The remainder of the monocytes present during the early hours of the reaction could not be differentiated from those of the circulating blood. During the first 24 hours the number containing dye increased very slowly, but between 24 and 48 hours the increase was much more rapid, and the cells containing the dye were larger; the giant macrophages so characteristic of subacute and chronic inflammatory reactions of the serous cavities appearing on the third or fourth day. It is evident that the cells usually found after an irritation can not all be cells which have been previously vitally stained in the tissues, the clasmatocytes; because the majority of the early cells of the exudate do not contain any vital dye (except in those experiments in which the blood-cells were vitally stained). And unless there are many errors in the work of the large group of authors who have studied the effect of the vital dyes on the clasmatocytes, all of these cells are usually stained. Further it is most unlikely that division of the stained cells could give rise to any new cells which would be absolutely free from dye. Kiyono (26) has found that no dye is lost by the cell undergoing mitotic division, the two daughter cells each containing about one-half the original amount of dye. If on the other hand the cells are elements which have emigrated from the blood-stream or are derived from the serosal lining cells they would contain no dye. Pappenheim in studying this question with the same technique, concludes that they are either preformed clasmatocytes or are derived from a primitive cell in the tissues having hematopoietic potentialities, and he denies that even the earliest monocytes appearing in the exudate are derived from the blood-stream. Lippman and Plesch, Szesci, Schott, etc., agree with Pappenheim in excluding emigration, but derive them in particular from the serosal lining cells.

In my experiments the evidence on the question of emigration is divided. In the experiments, in which the cells of the blood-stream were found to be stained, the majority of the exudative cells were likewise stained, while in the remainder of the experiments where the blood-cells were unstained, practically all of the cells of the exudate appearing early contained no dye. The conclusion has therefore been reached both from my own experiments and from an analysis of the literature

that all the polymorphonuclear neutrophiles, part of the eosinophiles, and part of the monocytes, appearing in the exudate during the early stages of an inflammation are derived by emigration from the blood-stream, while part of the monocytes and perhaps some of the eosinophiles are derived autochthonously.

Evidence concerning serosal lining cells. To what extent do the lining cells of the serous cavities participate in the formation of the cells of the exudates following any type of stimulation in these areas? This subject is one of very great general interest because it involves the question of the specificity of cell-types, and the extent to which any cell having already been differentiated up to a certain point is capable of reverting to a more primitive state and beginning anew its differentiation along some other line. The views that have finally become crystallized are the following: *a*, that serosal cells desquamate as typical flat cells and then act as primitive stem-cells capable of undergoing differentiation through several stages to become monocytes or special granular leucocytes. *b*, That these cells swell up as the result of irritation, become changed in shape, put out processes, detach themselves from the basement membrane and continue life as free, living, typical, highly specialized macrophages. *c*, That serosal cells are incapable of becoming free, living exudate-cells but can during inflammatory reactions act as vasoformative cells or can change to fibroblasts. *d*, That they are highly specific lining cells capable of no further differentiation, and that any of them desquamating are injured or degenerated after becoming free; and that the changes taking place in them during an irritation represent some exaggeration or modification of normal functions and attributes. The theory that the flat, normal, serosal lining cells desquamate immediately upon being irritated and later, while free in the cavity, undergo differentiation into characteristic exudate-cells, is based wholly upon morphological data. Many observers have described the changes which take place in serosal lining cells during inflammation. Cornil and Ranvier (8) first noted these changes. Cornil (7) describes great proliferation of the serosal lining cells and the formation of a syncytial network by the lining cells and fibroblasts indiscriminately. He considers that they also take part in the formation of new blood-vessels.

Others consider these changes in the serosal cells as evidence of their participation in the formation of exudate-cells, but on the other hand Pappenheim, Evans and others deny the participation of the serosal mesothelium in the formation of exudate-cells on the basis of excellent

evidence. It is well known that Pappenheim has carried cell differentiation on morphological and tinctorial grounds to the highest point of accuracy, and it is on these grounds that he feels the serosal cells do not enter into this reaction. He finds the serosal cells generally are unstained by vital dyes, and in addition to these observations he holds that the cells lining the pericardial cavity and the subarachnoidal spaces are different in their derivation and characters from those of the peritoneal and pleural cavities, yet an exactly analogous process takes place in these regions; so that Pappenheim refers the source of the exudate-cells to the preformed resting-wandering cells and cells derived from a primitive hematopoietic stem. Evans comes to a similar conclusion regarding the lack of serosal cell participation in the formation of the histiocytes of the exudate on the basis of their reaction to vital dyes. While the histiocytes of the exudate always show very heavy inclosures of trypan blue and other vital stains, the serosal cells have only a few, fine droplets, and this difference in reaction he thinks is sufficient to rule out the definite relationship postulated by Schott, Lippman and Plesch, and others. Pappenheim's statement that the peritoneal mesothelium differs from that of other localities does not seem to me to be in its entirety an admirable argument in the light of more recent work. His (25) and Van der Stricht (58) have shown that the primitive coelom is derived from the mesoderm by the flattening out and separation of two rows of cells, the cavity so formed later becoming the coelom and eventually dividing to form the pleural, peritoneal and pericardial cavities. The increase in size of these cavities, demanding an increase in the number of cells covering the surfaces, is attained by a rapid proliferation of the primitive surface-cells. The cerebrospinal spaces originate quite differently, as has been shown by Weed (59). In his investigation it became apparent that these spaces are formed by the enlarging of tissue-spaces through the breaking of the cellular bridges between the elements of the general primitive mesenchymal syncytium. It is thus evident that the cells lining these spaces cannot be considered as comparable to the coelomic derivatives and must be regarded as a separate strain of cells, such as endothelium, fibroblasts and other groups. The question regarding the reactions of these strains of cells, as such, will be referred to in the general discussion.

The two questions which now require answering are: *a*, do mesothelial cells desquamate and then undergo further development; or, *b*, do they differentiate in situ and desquamate as fully developed exudate cells of the cavity? In designating certain free cells of the exudate as serosal

cells, the observers have in general depended upon specific staining reactions of fixed smears. This undoubtedly accounts for much of the discrepancy which exists in the different descriptions. In the course of my experiments it soon became apparent that other preparations than sections and fixed smears were essential before enough criteria could be established to determine the relationship of serosal lining cells to the cells of the exudate.

Modern work has shown how important it is to study cells while living, and in a state as nearly approximating the normal as it is possible to obtain. In order to meet these conditions cells were carefully scraped off from any serosal surface, using a sharp knife, great care being taken not to injure the cells more than absolutely necessary to remove them. Sufficient fluid was generally removed from the scraped surface to act as a medium. The material obtained was put at once on a warm cover-slip and then inverted on a warm slide, and immediately rimmed with vaseline. These preparations were placed in an incubator containing a microscope and regulated to remain at 37°. In this way it was possible to examine every specimen within one minute after removal from the living animal, and they could be kept at body temperature and observed at any desired intervals. This technique has been followed throughout except where specific need for variation was found; the principal change being the addition of supravital staining with neutral red and brilliant cresyl blue. Pappenheim's method has been found most satisfactory; he used a saturated alcoholic solution of the dye, and applied it by passing a glass rod dipped in the solution across the clean surface of a warm slide. The evaporation of the alcohol leaves a very delicate, dry film of dye. These slides were used in exactly the same manner as described above; if the film has been correctly made the dye will be sufficient to stain the living cell without any apparent injury. In an effort to obtain the most advantageous medium for keeping these cells normal, they were studied both in Locke and in Locke-Lewis solutions, with and without the addition of neutral red, in blood-plasma of the same animal, and in freshly drawn drops of blood. Smears were also made in the usual way and fixed in formalin-vapor and in osmic-vapor.

Serosal cells from the body-wall of a rabbit, stained intravenously with trypan blue, when studied by this technique were in every case large, irregular, flat cells with vesicular nuclei. The cytoplasm contained a few very fine blue granules disposed in an irregular rosette around the nucleus. When these cells were stained in neutral red and studied immediately after being removed, they showed a tinge of red or purple in

the granules before seen as blue. Occasionally one or two fine red droplets were also to be seen, but these usually appeared only after irritation. On standing for 45 to 60 minutes a few more neutral red bodies became apparent, but this increase was very slight. The cytoplasm usually presented an appearance which was suggestive of granulation, but in which the individual granules could not actually be made out, except in isolated instances. The cytoplasm resembled ground glass in appearance but tended slightly to a more foamy character. When individual granules were present they were highly refractive and uniform in size.

None of these granules, either stained or unstained, can be considered as degeneration phenomena developing after removal of the cells from the living animal, because when they were observed immediately after removal, only in rare instances did they increase in number on standing. Moreover it is certain that the trypan blue was absorbed and stored during the normal life of the cell, and since the neutral red appears in the same areas these cannot be considered as degeneration vacuoles.

When an irritant was applied to the peritoneal surfaces, e.g., the injection of a suspension of erythrocytes, there were immediately found in the exudate a few cells, which were unquestionably desquamated serosal cells, that had become loosened from their cellular connections before undergoing any change. Their content in trypan blue and the arrangement of the dye concretions were entirely similar to those found in cells removed from the body-wall and other serosal surfaces, except that an occasional cell showed evidences of degeneration, nuclear changes, and sometimes a few, irregular, neutral red vacuoles containing no blue. Some of these injured cells had slightly tinged nuclei and other signs of a moribund or dying condition.

There is no evidence of transition between the normal serosal cells which have desquamated and the phagocytic monocytes of the exudate. That the normal serosal cells are phagocytic in the sense of passing substances through their bodies has been shown by Shipley and Cunningham (52) and by Cunningham (9), but that they store such phagocytized material except in very small amounts has never been shown. Schott describes the serosal cells as phagocytizing erythrocytes and leucocytes, but he also admits that he is unable to differentiate the various cells of the omentum, and considers them so much alike as to be practically the same type as far as physiological reactions and capabilities are concerned. I have seen serosal cells with phagocytic enclosures, but such enclosures are rare, and correspond to similar phagocytic

reactions on the part of other highly differentiated cells, such as fibroblasts and blood vascular endothelium.

When these serosal cells were examined 48 hours after the injection of any mildly irritating substance, erythrocyte-suspension, hypertonic saline, etc., a definite change was observed. The amount of trypan blue was unchanged, the amount of neutral red taken up was the same, but the clear refractive granules were increased in number, and the ground glass appearance had changed to a definite granular one. The cells had increased somewhat in size and the nuclei were plumper. After four injections at 24-hour intervals the cell-bodies were found to contain masses of these definite, highly refractive granules which were usually clumped in patches, but sometimes formed a rosette about the nucleus. The cells covering the body-wall were rounded up, their nuclei had become large and vesicular, a rosette of trypan blue granules surrounded the nucleus and patches of these characteristic, highly refractive granules could be seen in the cytoplasm, the cell-bodies being sometimes almost filled with them. When the cells were viewed from the surface of the cavity, they seemed entirely filled with these granules, if from a lateral point usually only one-half was full. It was found that the content of these cells in trypan blue could be modified in several ways. If the animal received no vital dye during the time that the irritation was taking place only the fine blue droplets, which have been described for the normal cell, were found. If these cells were also supravitally stained with neutral red some additional fine red droplets were found in the perinuclear rosette. On the other hand if the animal received repeated doses of trypan blue intravenously during the time that the injections were being given into the peritoneal or pleural cavities, the cells contained more of the fine droplets of blue than normal cells, but usually there were some few additional vacuoles which stained with neutral red alone. Finally in many experiments the trypan blue was given intraperitoneally in order to bring these cells into the closest possible contact with the dye and thus furnish them every opportunity of taking it up while they were undergoing the changes described above. In these animals the enlarged serosal cells always contained considerably more trypan blue than they did in any of the others, but always in the same fine droplets though often more widely disseminated thoughout the cytoplasm, and here there were no additional vacuoles which stained supravitally with neutral red. The highly refractive granules described above, which are so characteristic of the irritated serosal cells, are important in this connection specifically, because they assist us very

materially in recognizing the serosal cells which have rounded up and desquamated.

What has been determined regarding the chemical nature and physiological significance of these highly refractive granules in the life of these particular cells will be described and discussed in the report referred to in the introduction. Here all that it is necessary to give is a brief summary of the reactions which they give to the usual histological reagents. These granules do not stain with any of the vital dyes so far employed; they are very easily destroyed by fixation and are soluble in most of the usual reagents used in histological technique. They are destroyed immediately by acetic acid, sublimate, alcohol, acetone, formalin vapor and all the usual combination fixatives; Zenker, Carnoy, Bensley, etc. In osmic acid (2 per cent aqueous solution), if the coverslips are floated on the surface and then washed in water, allowed to dry, and studied unmounted, the granules appear a faint grey-brown; if mounted in balsam the granules are slowly dissolved, leaving vacuolated cytoplasm. A similar, but more rapid solution takes place in alcohol. All attempts to stain these granules after osmic fixation have failed, the reagents used either dissolving the granules or leaving them unchanged. In fresh preparations these granules stain a faint pink with Sudan III, but do not stain with nile blue.

When the injections were repeated at 24- or 48-hour intervals for a week or 10 days, the serosal cells hypertrophied to their maximum state, and further injection caused desquamation and proliferation of the remaining cells, but no further changes in type. These cells even after they have reached the maximum hypertrophy may return to normal if the injections be stopped; so it seems evident that if the cells have undergone any injury it must be considered as certainly within the limits of recovery.

The general appearance of serosal cells from the body-wall during the maximum hypertrophy has been given, but it is essential to mention certain variations from this general description which occur in specific areas. The diaphragmatic cells were found to be quite similar to those of the body-wall except that they increased in size much more rapidly, but did not contain quite so many highly refractive granules. The splenic mesothelium was particularly interesting because during any irritation the cells rapidly became cuboidal and finally cylindrical. The nuclei were located about midway between the base and apex of the cell and in general morphology the cells suggested the appearance of the normal intestinal epithelium. The trypan blue was almost always

found in a small clump of vacuoles situated near the nucleus in the base of the cell, and here also the neutral red vacuoles were to be found. Patches of the highly refractive granules were found in the cytoplasm of these cells, usually in the same part that contained the trypan blue vacuoles.

Characteristic, desquamated, serosal lining cells can be seen in the serous exudates at any time during a chronic irritation. As has been pointed out these cells can be easily distinguished from the other cells of the exudate, by their sparse and typically distributed trypan blue concretions, and the numerous refractive granules which have been described above. In addition they have characteristic vesicular nuclei when stained with a Romanowsky stain, and a reticular or foamy cytoplasm marking the areas from which the highly refractive droplets have been dissolved. These desquamated serosal cells degenerate rapidly in the exudate and in consequence only a few having the typical appearance just described are to be seen at any time, while those which show signs of degeneration are more numerous. There are two changes which are particularly noteworthy in the degeneration of these cells. The nuclei become swollen and edematous and seldom show any signs of pycnosis, and the vacuoles become considerably larger and more irregular. It was found that when an animal had received daily doses of trypan blue intraperitoneally for a week or longer, the exudate contained a group of very large cells with medium sized vacuoles colored pale blue, intermixed with very numerous highly refractive granules. These were serosal cells in a moderately advanced stage of degeneration. When the exudate was studied on a warm neutral red slide, it was found that these cells took up the red brilliantly in the vacuoles that were previously pale blue, and the clear, refractive granules were to be seen still unstained but in active brownian movement between the deeply stained vacuoles. In a few minutes the nuclei took on a tinge of pink and very soon were stained a definite red, showing that the cell was dead. In these specimens the true clasmatocytes were present in every case and contained a great many vacuoles of different sizes all of which were stained deeply by the trypan blue; these cells took up neutral red also, but continued to live for hours without any evidence of a pink staining of the nucleus. If the animal had received the vital dye intravenously and had had some other type of irritant injected into the peritoneal cavity, then the degenerating serosal cells more closely simulated the normal, until stained by neutral red when a group of medium sized vacuoles were discovered which remained unstained by the trypan blue; the death of

the cell took place as described above. All intervening stages of degeneration between the normal and moribund cells were found.

Evidence concerning the cells of the omentum. The omentum deserves especial attention both because it has been so often cited as the source of free cells, and because the serosal cells as well as the clasmatocytes are most interesting and characteristic in this organ. Schott has described the omentum in fixed specimens, and Marchand in the living, but their interpretations are insufficient.

When a small bit of the omentum from a rabbit, which had been vitally stained intravenously and had received several intraperitoneal injections of washed, red blood-cells, was studied on a warm coverslip, mounted on a hollow-ground slide, a very interesting picture was found. Two types of cells were immediately seen, one loaded with trypan blue and the other containing only a few fine perinuclear and scattered blue inclusions, but very many highly refractive, unstained granules. All of the cells of the first type had certain similarities; they were all very deeply stained with blue, the vacuoles containing it being of extremely varied sizes, from some very small ones to others almost as large as the nucleus. Their contour was smooth, and here and there blunt processes were to be seen, often containing a leucocyte or other phagocytized material. Some of these cells were stretched out to form very long, thin lines of blue, which often were found to reach one-half or two-thirds across the oil immersion field. When the spread of omentum was made on a coverslip which had a thin dry film of neutral red on it, the clasmatocytes on the surface became loaded with the red dye, so that the vacuoles previously colored blue were purple in color. The cells deeper in the meshwork remained unstained by the red so that the contrast was very striking. Some few of these cells were near enough to the surface to absorb a small amount of red dye, but not enough to stain them brilliantly. Most of these clasmatocytes were large cells with rounded cytoplasm but some were long and thin, others smaller in size but having all the general characteristics of macrophages.

The serosal cells appeared as large oval cells containing highly refractive granules in every way similar to those seen elsewhere. The pavement arrangement of these cells, so characteristic in the normal omentum, had become changed in these experimental animals. Some of the cells of the covering layer had rounded up and been desquamated, others had rounded up and remained attached, while a few, not so much changed, were difficult to distinguish from fibroblasts. The cells which remained, whether they were relatively flat and apparently sessile, or rounded up as

though about to desquamate, all reacted characteristically to the vital dyes. A few, fine blue, red or purple droplets were scattered among the refractive, unstained granules and were often grouped in little clumps, sometimes near the nucleus. The clasmatocytes and serosal cells were distinguished by this staining reaction, their granular content, and the contour of their cell-bodies. The clasmatocytes were large or medium sized cells, with blunt rounded processes and relatively smooth contour and contained masses of stained vacuoles, and very few granules of other kinds; while the serosal cells had outlines of a more irregular, indented character, showed only a few fine, vitally stained vacuoles, and contained masses of highly refractive granules.

When fixed in osmic vapor, stained in carmine, dehydrated and cleared, the serosal cells were seen as oval or rounded structures with a reticulated cytoplasm from which the granules had been dissolved leaving a grey tint to the cell. The grey color was undoubtedly due to the staining of the envelope of the granule by the osmic acid; this greyish reticulated cytoplasm marked out the bounderies of the cells more sharply than they appear in the living specimens. The nuclei were large and oval and stained pink with carmine.

Evidence concerning clasmatocytes. Beginning at about 30 hours after inflammation was induced in animals that had been vitally stained with trypan blue, and becoming more and more numerous there appeared in the serosal exudates a group of large, heavily stained cells. The origin of these cells, which has been so much disputed, can be ascribed, on the evidence afforded by my experiments, to only one source, the preëxisting clasmatocytes of the neighboring subserosal tissues. The omentum is a great store-house for these cells, where they are distributed particularly along the blood-vessels and in the *tâches laiteuses*, but are present everywhere. Buxton and Torrey (3) describe these cells as macrophages from the peritoneal exudate, which have gained entrance to the tissues and often flatten out along the vessels to become their so-called "trailer cells." On the contrary it is certain that these cells are normally present in the omentum, and are identical with Marchand's adventitial cells and the clamatocytes of most authors. As soon as any irritant stimulates the serous membranes these cells migrate toward the surface and pass into the exudate. Of course many of them may remain adherent to the omental or other serous surfaces because of the formation of small amounts of fibrin. It is interesting to compare the time required for these cells to reach the cavity with Maximow's findings in the case of tissue inflammation. In his experiments the clasmatocytes appeared

after about 20 hours, while in mine the time of the first appearance was obviously much more difficult to establish exactly, but was found to be between 30 and 36 hours.

The clasmatocytes of the exudate are most characteristic in their reactions to vital stains and to injected material. The description given of their appearance while still inhabiting the omentum is in many ways similar to that which they present as free cells of the exudate, but in the exudate they have a few characteristics which are different. They are usually smaller, more compact and more rounded in form; the processes which they put out very slowly are sometimes blunt pseudopodia and sometimes fine flagella-like processes. These latter have never been seen to form while the cells are being observed but are found most often in the clasmatocytes of very copious exudates. Whether they have any relation to the osmotic pressure or other properties of the surrounding medium is not known. Sabin (51) finds them in the developing monocytes of the embryo-chick, and describes them as waving about and keeping the fluid disturbed while the cell itself has an exceedingly slow form of locomotion.

When afforded every opportunity, i.e., by intraperitoneal injections, the clasmatocytes take up very great amounts of trypan blue without the ability of the cell for phagocytosis being interfered with. I have found that clasmatocytes which have been previously stained with trypan blue are still capable of phagocytizing eight or ten avian erythrocytes in a few hours. At times the blue vacuoles are so thickly massed that the nucleus can not be made out in the living cell, and only becomes apparent when stained with some red dye. The clasmatocytes of the exudate vary greatly in size, but are similar in their reaction to vital stains and other material which may be phagocytized. When fixed and stained with carmine their nuclei are relatively small, oval or round, sometimes indented, and always staining rather brilliantly in contrast to the pale nuclei of the serosal cells. With a Romanowsky stain they usually show no granules but practically every one contains some kind of cellular debris, the remains of phagocytized cells. Their nuclear characteristics and the varying reactions of their cytoplasm to the large number of hematological stains have been given in minute detail and with great accuracy by Pappenheim and Fukushi (45) and by Szecsi (56) and need not be repeated here. Finally the ordinary tinctorial reactions are misleading because they evidence many types of cells which can be so easily proved, by direct observation and by experimental examination, to be merely stages in the life history of the same

cell, the clasmatocyte. And these staining peculiarities are particularly marked in those cases, such as inflammatory exudates, where in addition degenerating cells may be found; it is obvious that many clasmatocytes must be injured and die as the result of conditions in the exudates. When a clasmatocyte is injured and undergoes degeneration it does not present quite the same picture as the desquamated and dying serosal cells because it has many more irregular vacuoles and the nucleus usually becomes pycnotic. Besides the far greater amount of trypan blue totally prevents any mistakes in this regard, once the typical serosal cell has been seen and recognized in all the stages from the freshly desquamated cell to the moribund, dying or dead cell. Mitotic division is very common in the clasmatocytes of the exudate; this has been observed and described by very many authors, and there are no particulars which can be added here. Finally it must be noted that there are a few, scattered, highly refractive granules to be seen in many of the free clasmatocytes of the exudate, and in those of the omentum as well. These granules react in a similar manner and have the same appearance as those described for the serosal cells, but have never been seen except as very sparse and scattered structures.

So many workers have emphasized the omentum as the most important source of the cells appearing in the inflammatory exudate that it seemed necessary to study the reactions of animals without omenta. Cats were used for these experiments. To accomplish this the omentum was removed by a sterile operation, leaving only that part of it which carries the splenic vessels; the animal was allowed to recover completely and subsequently the peritoneal irritation was carried out as usual.

The differences between the exudates found in these animals and those of control animals were very slight. The clasmatocytes of the exudates did not differ in any appreciable way from those of the control experiments either in size, staining reactions, or phagocytic capability. But there was a change in the ratio between the number of clasmatocytes and desquamated serosal lining cells, the former being decreased the latter increased in number. The desquamated serosal cells were wholly similar to those of the usual exudate, but they had greater tendencies to appear in clumps and small sheets which showed all the usual signs of degeneration.

It is thus evident that the omentum has some tendency to protect the remainder of the serosal linings from the irritant while it furnishes an appreciable quota of clasmatocytes. But these experiments also demonstrate that the omentum is in no sense the sole, or even the predominant source of the free cells of the exudate.

GENERAL DISCUSSION

The precise origin of the mononuclear cells of serosal exudates has been discussed in the preceding pages, but it seems essential to review as well, what information has been obtained in regard to the genetic relation of these cells, and similar cells of blood and connective tissue, to certain strains of fixed cells.

Since the first work of Ehrlich (14) on the classification of the blood-cells, the monophyletic and polyphyletic schools have each been engaged in elaborating technique and describing morphological criteria in support of their respective tenets. Recently much help has been brought to the clarification of this most complicated tangle by the brilliant studies of Sabin (50), (51). She has found that in the chick the formation of the earliest blood-vessels takes place through differentiation of a new cell, the angioblast, from the primitive mesenchyme. These angioblasts are characterized by their tendency to form syncytial masses, the center of which liquefies to form the first blood-plasma, while the margins flatten out to form the earliest endothelium. New angioblasts continue to differentiate from mesenchyme, while those already formed grow by cell division and sprouting. They join each other by the meeting and fusion of sprouts, which like the rest of the angioblasts rapidly hollow out to form primitive vessels. This primitive endothelium continues to grow by sprouting, and joins to new angioblasts or other growing endothelium.

In further studies she finds that this primitive endothelium has additional capabilities; on the second day cells bud off into the lumen of the young vessels, and there by proliferation form blood-islands, from which primitive erythrocytes become detached and are swept into the circulation. At a little later stage, i.e., on the third day, in scattered areas the endothelial cells divide to form rows of extravascular cells which develop rapidly into wholly characteristic clasmatocytes. During this same time single new cells are often formed from the endothelium and after becoming quite large they are detached intravascularly and so become free in the circulating blood. These cells she identifies as the monocytes of the circulation, and considers them entirely identical with the extravascular clasmatocytes. The development of the granular leucocytes takes place extravascularly and she believes that they come either directly from mesenchyme or from some special derivatives. She obtains no evidence of any interrelation of these cell stems. Monocytes are never seen to give rise to granulocytes, nor is there any evidence that monocytes or clasmatocytes arise in any other way save from

endothelium. The indications are strongly in favor of a very early acquisition of specific properties which do not permit any return to more primitive types.

In the adult the question of the origin of monocytes from endothelium has been difficult to approach. Mallory (33) (34) found in studying the pathological processes of typhoid fever that great numbers of large characteristic cells are formed in lymph-glands and spleen, and these he attributes to the proliferation of reticulo-endothelium. He describes the reticulum as forming a branched framework in the lymph-glands and spleen, and thinks that endothelial cells line not only the sinuses, but the reticular strands as well. And while he emphasizes the part of the endothelium in the production of the so-called endothelial leucocytes, he thinks that the endothelium and reticulum are practically indistinguishable. This question of the relationship of reticular cells and endothelial cells to the phagocytic mononuclears has also been approached by Aschoff and Kiyono (1) and by Kiyono (26), (27). Kiyono describes the reticular cells of spleen and lymph-gland as taking up moderate amounts of carmine in vitally stained animals, while the endothelial cells of the sinuses store it much more abundantly. He describes the general vascular endothelium as containing little or no carmine, but considers that all endothelium, together with reticulum and the histiocytes have a "definite biological relationship." Nevertheless he thinks the histiocytes are derived principally from the reticulum, although he admits the possibility that the sinus endothelium may also participate. With regard to the possibility of other cells taking any part in the production of histiocytes, he is confident that the fibroblast is a highly differentiated cell-type, which may wander out into inflammatory areas, but never looses its peculiar biological and morphological characteristics, while in regard to the vascular endothelium and serosal lining cells, he finds evidence of their proliferating to reproduce only cells of the same type and function.

Downey (12) takes the opposite view to that of Mallory with regard to the relationship between reticulum, endothelium and histiocytes. The large cells which are seen free in the meshes of the recticular network he derives from reticulum, and finds every transition between the two. He does not agree with Mallory regarding the existence of endothelium lining the meshes of the reticulum, but thinks these cells are flattened, reticular cells. Further he says: "Since there is no endothelium covering the reticular strands or lining the sinuses we are hardly justified in naming the large cells which are cut off from the reticulum 'endothelial

leucocytes'. Whether such cells may also be derived from the endothelial cells lining lymph and blood vessels, as claimed by Mallory, still remains to be demonstrated. Theoretically there is nothing against such a view, since numerous investigators, including the writer, have shown that similar cells may be derived from the covering cells of the omentum and serous layers generally. However, Weidenreich and Schott believe that these covering cells are merely flattened surface fibroblasts. If this view is correct these covering fibroblasts would not be very different from the reticulum cells of the lymph-nodes." Downey goes further and derives similar mononuclears from fibroblasts in such locations as the connective tissues of the liver, walls of blood-vessels, etc., and even admits their derivation from lymphocytes, a conclusion denied by the majority of investigators including Goldmann, Evans, Aschoff, Kiyono, etc.

Two pupils of Mallory have contributed further evidence with regard to the derivation of the mononuclear cells of blood and tissues. McJunkin (30) holds that all phagocytic mononuclears of the blood are derived from blood vascular endothelium, that of spleen, lymph-gland, liver, bone-marrow, and the capillary walls in general. In one place he says: "There is a tendency to recognize a close relationship or identity of cells to which such terms as adventitial cells, gland connective tissue cells, areolar connective tissue cells, reticulum cells, etc., and the fibroblast. Certainly proof that is positive and easily obtained shows that such extravascular cells do not ingest by phagocytosis and do not give rise to phagocytes." This statement is certainly not in accord with the ideas of the vast majority of investigators, particularly with regard to the adventitial cell or clasmatocyte, which is usually regarded as the most active of phagocytes. McJunkin used for injection a suspension of carbon particles in a citrate-gelatin solution. By the addition of gelatin he was able to obtain a very fine suspension, and by the use of citrate prevented the polymorphonuclear leucocytes from taking up the ink. McJunkin believes that the carbon particles, which he obtains by filtration of his suspension, are phagocytized very specifically by endothelium and endothelial derivatives. As the citrate prevents its being taken up by the polymorphonuclear leucocytes, he claims to have found a specific stain for the other two groups, and on this basis concludes that the phagocytic, mononuclear cells of the blood which take up these carbon particles readily, are derived exclusively from endothelium.

Foot (18) applied McJunkin's technique to the study of inflammatory reactions and obtained comparable results. He studied the re-

actions of subcutaneous tissues around injected agar in animals stained intraperitoneally with trypan blue, and intravenously with india ink, and concludes that the majority if not all of the free macrophages of inflammatory connective tissues are of local endothelial origin. In later experiments he studied the formation of endothelioid cells in tuberculosis and concludes on similar grounds, i.e., the content of the cells in carbon granules, that they are of endothelial origin. Although no experimental evidence is reported, the following statement is made which certainly does not agree with the other observations on the vital staining of the serous cells. "I have pointed out that the mesothelial cells of the omentum—which are undoubtedly phagocytic and probably perform the same function for the serous cavities as the endothelial cells do for the loose connective tissue—are deeply stained by trypan blue." This claim for the vital staining of the mesothelium and its participation in the production of true phagocytic cells finds no support in my experimental results.

In general the studies reviewed here have tended to establish the origin of the large mononuclear cells of the blood from certain fixed tissue-cells. In the embryo it seems certain that they are derived from endothelium. In the adult their origin has become localized to those areas, i.e., spleen and lymph-glands, where reticulum and endothelium are distinguished only with difficulty from each other and opinions are still divided as to which of these cells is the progenitor of the circulating mononuclear. Foot's conclusion that all phagocytic cells of inflammatory reactions, as well as the phagocytic mononuclears of the blood, are derived from the general capillary endothelium, suggests that these cells may retain the developmental capability which Sabin has shown that embryonic endothelium possesses. The criteria upon which McJunkin and Foot base their conclusions, while undoubtedly valuable, do not seem to establish sufficiently this genetic relationship for the adult. In the production of exudates in the peritoneal cavity I have obtained no evidence either for or against the participation of endothelium of neighboring capillaries, e.g., those of mesentery and omentum, in the formation of exudative cells. Some observations of my own, made in a different connection, suggest the hypothesis that if the circulation be cut off from a group of capillaries, the endothelial cells of which still obtain sufficient nourishment to prevent cell-death, these cells may undergo a cataplastic reversion to the syncytial angioblastic or embryonic endothelial type, with subsequent differentiation into clasmatocytes. This theory is supported by some observations of Macklin and Macklin (32)

who found that areas of endothelium in new-formed capillaries appear to become transformed into clasmatocytes.

Turning now to the fibroblast: it is essential to consider the relation which this important cell bears to the monocyte, the clasmatocyte, and the serosal cell. To Maximow belongs the credit of first differentiating the clasmatocyte from the fibroblast, and of determining their relative rates of motility toward an inflammatory reaction. The careful studies of Clark and Clark (4), (5), (6) on the reactions of the fibroblasts and clasmatocytes in the tadpole's tail to inflammatory agents have aided materially in finally establishing the specificity of both types. Evans and Scott (17) have given us the final word on the separation of these two cells as a result of their studies with two hundred benzidene dyes in which they catalogued the characteristic reactions of each cell to the different dyes in acute and chronic staining. Practically all the work that has been done since Maximow has strengthened his idea of the specificity of the fibroblast, and this cell may now be safely set aside as a highly specialized cell with a specific function. That the fibroblast can round up, move slowly toward an inflamed area, even become phagocytic to some extent is well known; but it remains a fibroblast and has definite functions. These functions may become suppressed or even exaggerated by inflammatory reactions, but that they become changed to a new type seems most unlikely; though it must always be held in mind that we know practically nothing regarding the real physiology of the great group of free cells of the body.

Mallory (35) suggests, in a recent paper, that the cells lining the subarachnoid spaces are not a specifically differentiated group of cells, but are flattened fibroblasts. Essick (15) on the other hand concludes, after studying the reaction of these cells to injected red blood-cells, that they increase in size, round up, and desquamate to form free phagocytic cells in the cerebrospinal fluid. Since it seems to be well established that there is no real transition between fibroblasts and clasmatocytes, the cells lining the arachnoidal spaces probably retain sufficient embryonic characteristics to be able to differentiate into new cell-types.

Some effort must be made to draw a general conclusion from the experimental data which have been briefly reviewed here. There are great groups of cells which have been differentiated from the primitive mesoderm and have developed certain special characteristics in response to the varying needs of the organism. All of these cells retain some of of the more primitive reactions of the mesoderm, and some of them retain sufficient of these characteristics to be able, under stimulation, to

develop into more highly differentiated cells. Those cells which almost certainly retain the capability of forming new cells of a more highly specialized character are the reticulum and endothelium of the sinuses of spleen and lymph-gland. On the other hand it is equally certain that the fibroblast, the clasmatocyte and the serosal lining cell can not undergo any such differentiation. With regard to the formation of free cells from the general vascular endothelium and the lining cells of the subarachnoid, much evidence has been presented on both sides, but it does not seem sufficient as yet to define the developmental potentialities which these cells retain in the adult state.

BIBLIOGRAPHY

(1) Aschoff and Kiyono: Folia Haemat., 1913, xv, 383.
(2) von Büngner: Beitr. z. path. Anat. u. allg. Path., 1896, xix, 33.
(3) Buxton and Torrey: Journ. Med. Research, 1906, xv, 55.
(4) Clark and Clark: Anat. Record, 1918, xv, 231.
(5) Clark and Clark: Amer. Journ. Anat., 1920, xxvii, 221.
(6) Clark and Clark: Anat. Record, 1918, xv, 151.
(7) Cornil: Arch. Med. Exper. d'Anat. Path., 1897, s 1, ix, 9.
(8) Cornil and Ranvier: Manuel d'histologie pathologique, Paris, 1869, 76.
(9) Cunningham: Anat. Record, 1920, xviii, 229.
(10) Downey: Anat. Record, 1917, xii, 429.
(11) Downey: Anat. Record, 1918, xv, 103.
(12) Downey: Anat. Record, 1915, ix, 73.
(13) Drinker and Shaw: Journ. Exper. Med., 1921, xxxiii, 77.
(14) Ehrlich: Farbenanalytische Untersuchungen zur Histologie und Klinik des Blutes, August Hirschwal, 1891.
(15) Essick: Contrib. Embry. no. 42, vol. ix, Carnegie Inst. of Wash. Publ. 272, 1920.
(16) Evans: This Journal, 1915, xxxvii, 243.
(17) Evans and Scott: Contrib. to Embry. no. 47, vol. x, Carnegie Inst. of Wash. Publ. 273, 1920.
(18) Foot: Journ. Med. Research, 1919, xl, 353.
(19) Hannemann: Zeitschr. f. Exper. Path. u. Therap., 1920, xxi, 28.
(20) Harvey: Amer. Journ. Anat., 1906–07, vi, 207.
(21) Heinz: Virchow's Arch., 1902, clxvii, 161.
(22) Heinz: Virchow's Arch., 1900, clx, 365.
(23) Heinz: Virchow's Arch., cli, 45.
(24) Helly: Beitr. z. path. Anat. u. allg. Path., 1905, xxxvii, 171.
(25) His: Abhandl. d. Math-phys., Cl. d. k. sachs. Gesellsch. d. Wissensch., 1900, xxvi, 171.
(26) Kiyono: Die Vitale Karminspeicherung, 1914, Jena, Gustav Fischer.
(27) Kiyono: Folia Haemat., 1914–18, xviii, 149.
(28) Lippman and Plesch: Deutsch. Arch. f. Klin. Med., 1915–16, xcviii, 283.

(29) Lippman and Plesch: Deutsch. Med. Wochenschr., 1913, xxxix, 1395.
(30) McJunkin: Amer. Journ. Anat., 1919, xxv, 27.
(31) Mass and Plesch: Zeitschr. f. Exper. Path. u. Therap., 1912, xii. 85.
(32) Macklin and Macklin: Arch. Neurol. and Psychiatry, 1920, iii, 353.
(33) Mallory: Principles of pathological histology, 1916, Philadelphia.
(34) Mallory: Journ. Exper. Med., 1898, iii, 611.
(35) Mallory: Journ. Med. Research, 1920, xli, 349.
(36) Marchand: Sitzungsb. d. Gesellsch. z. Marburg, 1897, No. 3, 29.
(37) Marchand: Sitzungsb. d. Gesellsch. z. Beforderung, d. ges. Natur., 1899, No. 6.
(38) Marchand: Verh. d. Deutsch. Path., Gesellsch., 1901, iv, Tagung.
(39) Marchand: Verh. d. Deutsch. Path. Ges., 1899, i.
(40) Maximow: Folia Haemat., 1907, iv, T. i., 611.
(41) Maximow: Folia Haemat., 1909, viii, 125.
(42) Maximow: Arch. f. mikr. Anat., 1909, lxxiii, 444.
(43) Maximow: Beitr. z. path. Anat. u. allg. Path., 1905, xxxviii, 301.
(44) Maximow: Arch. f. mikr. Anat., 1906, lxvii, 680.
(45) Pappenheim and Fukuschi: Folia Haemat., 1914, xvii, 258.
(46) Pappenheim: Centralbl. f. allg. Path. u. Anat., 1913, xxiv, 997.
(47) Pappenheim and Plesch: Zeitschr. f. Exper. Path. u. Therap., 1913, xii, 95.
(48) Pappenheim and Plesch: Folia Haemat., 1912–13, xiv, 1.
(49) Plesch, Karczog and Kutman: Zeitschr. f. Exper. Path. u. Therap., 1912, xii, 1.
(50) Sabin: Contrib. Embry. no. 36, vol. ix, Carnegie Inst. Publ. no. 272, 1920.
(51) Sabin: Johns Hopkins Hosp. Bull., 1921. xxxii, 314.
(52) Shipley and Cunningham: This Journal, 1916, xl, 75.
(53) Schridde: Centralbl. f. allg. Path. u. Path. Anat., 1912, xxiii, 902.
(54) Schridde: Studien und Fragen zur Entzundungslehre, Jena, 1910, G. Fischer.
(55) Schott: Arch. f. Mikr. Anat., 1909, lxxiv, 143.
(56) Szecsi: Folia Haemat., 1912, xiii, 1.
(57) Szecsi and Ewald: Folia Haemat., 1914, xvii, 167.
(58) Van Der Stricht: Compt. Rend. d. la Soc. d. Biol., 1895, s. 10, xii, 181.
(59) Weed: Contrib. to Embry. no. 14, vol. v, Carnegie Inst. Publ. no. 225, 1917.
(60) Weidenreich: Arch f. mikr. Anat., 1908, lxxii, 209.
(61) Weidenreich: Anat. Anzeiger, 1907, xxx, Verh. d. Anat. Ges., Wurzburg, 51.

THE INFLUENCE OF ADRENALIN ON METABOLISM IN ISOLATED SKELETAL MUSCLE

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Boothby and Sandiford (1) have reported a series of experiments on human subjects in which they demonstrate a marked increase in heat production from the subcutaneous injection of adrenalin chloride. They interpret these experiments as indicating a "specific dynamic action of adrenalin chloride on all the cells of the body causing them to metabolize more rapidly under its influence." Aub, Foreman and Bright (2) have demonstrated a reduction of basal metabolism in cats following adrenalectomy. These observations suggested to us the possibility that direct effects of adrenalin might be demonstrable on isolated tissue. Various observers, A. V. Hill (3), W. M. Fletcher (4), Eddy and Downs (5), have shown that metabolism proceeds in isolated muscle with the production of heat and the evolution of carbon dioxid. It is the influence of adrenalin upon this metabolism that we proposed to investigate.

Our method was that proposed by Haas (6) by which carbon dioxid evolution is estimated with the aid of indicators. Our modifications of Haas' procedure were such as to adapt it to our special requirements. In brief the technique was as follows: the excised frog's sartorious was used, utmost care being taken in every instance to perform the excision without damage to the muscle tissue. The excised muscle was weighed and then attached by silk sutures at the lower end to an L-hook extension at the bottom of a fine glass rod, and at the top to a light muscle lever. After the muscle was placed in position it was immersed in 4 cc. of Ringer's solution previously made neutral with a minute amount of sodium bicarbonate and to which had been added 8 drops of 0.02 per cent phenolsulphonephthalein solution. For a container we used a Wassermann tube of pyrex glass which had been cut down to a total capacity of 6 cc. The immersed muscle with its supporting rod raised

the level of the liquid so that little air space was left in the tube. The tip of the Wassermann tube was closed by a paraffined cork through whose middle a small glass tube extended to afford passage for the thread from the muscle to the recording lever. A drop of mineral oil placed in this tube eliminated the possibility of gaseous interchanges between the tube and the outside air while permitting a free motion of the thread connecting the muscle with the lever. In every experiment this preparation was made in duplicate, the two sartorious muscles from one frog being prepared as quickly as possible. The moment of immersion in the Ringer's solution was noted carefully as was the hydrogen ion concentration of the solution by comparison with a set of standard buffer indicator tubes. As soon as the color of the solution had definitely changed, a period requiring about 20 minutes in Ringer's solution at ordinary room temperatures, the exact time was noted and the muscle removed from the solution. The hydrogen ion concentration was again carefully determined immediately and the production of hydrogen ions per gram of muscle substance per hour computed.

Highly concordant values were given by the two muscles of the pair in nearly every control experiment, as will be seen from table 1, in which the results are set down. In each instance the muscles remained perfectly quiet throughout the period of experimentation, as was shown by having the tips of the writing levers in contact with a smoked drum and observing that no contractions were executed during the experimental period. Electrical stimulation at the end of the experiment showed the muscles to be still alive and highly irritable.

The room temperatures at which the control experiments were carried on ranged from a minimum of 16°C. to a maximum of 23.4°C. In order to find out how constant the acid production is in isolated frog's muscles, the actual figures were reduced to a standard temperature of 20°C., basing the calculation on the usual formula for the temperature coefficient of chemical reactions. The reduced figures are given in table 1. Reference to them shows clearly that with a few exceptions the acid production is at a satisfactorily uniform rate. The chief exceptions are the first test of February 12 and the experiment of March 1. Omitting these three non-concordant experiments, the average metabolism per gram of muscle per hour for this series, expressed as C_H, was $110 \pm 4.2 \times 10^{-3}$. The spread was from 66 to 196, the grouping about the mean being that called for by the normal probability curve, as shown by the fact that the central two sigma included 68 per cent of all the figures as demanded.

TABLE 1

Control experiments

DATE	TEMPERATURE	ACID PRODUCTION*	CALCULATED ACID PRODUCTION AT 20°	RATIO LARGER TO SMALLER OF PAIR
1921	°C.			
February 1	21.8	80	73	1.02
		79	72	
February 5	19.0	121	133	1.48
		82	90	
February 8	22.8	100	86	1.30
		76	66	
February 12	16.0	178	249	1.27
		140	196	
February 12	18.0	114	137	1.54
		74	89	
February 15	20.0	102	102	1.02
		100	100	
February 19	19.0	164	180	1.01
		165	182	
February 22	22.5	127	111	1.02
		124	109	
February 26	21.0	117	111	1.03
		114	108	
February 26	23.0	89	76	1.04
		86	73	
March 1	23.4	275	228	1.02
		280	232	
March 5	20.8	138	133	1.01
		140	134	
March 5	22.0	131	118	1.02
		129	116	
March 8	22.0	93	82	1.09
		83	75	
March 13	20.0	133	133	
		131	131	1.01
March 16	23.0	120	102	1.02
		118	100	

* The figures in columns 3 and 4 are to be multiplied by 10^{-8} to give the actual results.

This series of control experiments satisfied us that our technique was adequate for the detection of specific effects of adrenalin on metabolism and we accordingly proceeded to the experiments for which the investigation was designed. In these experiments the technique was precisely as above outlined except that one of the sartorious muscles of the pair instead of being immersed in Ringer's solution was immersed in a solution of adrenalin in Ringer's fluid. In making these solutions the dry adrenalin of Parke, Davis & Company was used. A stock solution of 10 mgm. of adrenalin in 100 cc. of Ringer's fluid was made up fresh at the beginning of each experiment and the desired concentrations made from this by further dilution with Ringer's fluid. The range of dilutions was from 1:5000 to 1:500,000. In order to be sure that our results were strictly comparable we allowed the acid production in both the control muscle and the muscle in adrenalin solution to proceed to the same color-change. The time required for bringing on this change was carefully noted, a shorter time signifying a more rapid production of carbon dioxid and, therefore, a more rapid metabolism. The reduction of acid production to terms of hourly production per gram of muscle substance gives a satisfactory basis of comparison.

As table 2 shows there is a marked augmentation of acid production in all strengths of adrenalin solution from 1:300,000 upward. The extent of the augmentation is indicated in the right-hand column of the table where the ratio of the acid production in adrenalin solution to the control is set down. It ranges from a virtual doubling at 1:300,000 to a fourfold increase at 1:20,000. That this latter represents the maximum effect is indicated by the fact that no greater increase occurred at a concentration of 1:10,000. Consistent increases in metabolism occurred in adrenalin solutions of 1:400,000 and 1:425,000. They do not, however, exceed the most discrepant results among our control pairs, as set down in table 1, and we would not lay stress upon them were it not that in solutions of 1:450,000 and 1:500,000 repeated experiments showed no adrenalin effect at all.

Duplicate experiments with given concentrations of adrenalin solution gave reasonably concordant results except in one of the experiments with 1:50,000, and in the series in which 1:200,000 was used. Our first two experiments with this concentration showed such divergent effects on metabolism that we carried out later three additional tests. The result was not satisfactory except as it suggests the influence of factors which we cannot at present evaluate.

TABLE 2

Adrenalin experiments

DATE	TEMPERATURE	ACID PRODUCTION	CALCULATED ACID PRODUCTION AT 20°	STRENGTH ADRENALIN SOLUTION	RATIO ADRENALIN EXPERIMENTS TO CONTROLS
1921	°C.				
June 4	19.0	122	134		
		563	619	1:5000	4.60
May 28	18.0	124	149		
		346	415	1:10,000	2.78
May 27	19.0	137	150		
		577	634	1:20,000	4.22
April 16	17.0	90	117		
		264	343	1:50,000	2.94
April 20	20.5	31.5	30.7		
		92	90	1:50,000	2.93
April 22	21.0	61	58		
		95	90	1:50,000	1.55
May 1	20.0	71.6	71.6		
		182	182	1:100,000	2.54
May 4	17.0	54.5	71		
		110	143	1:100,000	2.02
May 6	15.2	37.6	56		
		133	199	1:200,000	3.56
May 11	20.5	71	69		
		118	116	1:200,000	1.68
June 4	18.0	176	211		
		650	780	1:200,000	3.7
June 11	21.0	110	105		
		216	205	1:200,000	1.95
June 11	22.0	127	115		
		248	223	1:200,000	1.94
May 13	19.0	66	72		
		130	143	1:300,000	1.98
May 14	21.0	130	123		
		278	264	1:300,000	2.14
May 14	21.0	181	172		
		206	196	1:400,000	1.14
May 18	18.5	156	179		
		180	207	1:400,000	1.21

TABLE 2—*Concluded*

DATE	TEMPERATURE	ACID PRODUCTION	CALCULATED ACID PRODUCTION AT 20°	STRENGTH ADRENALIN SOLUTION	RATIO ADRENALIN EXPERIMENTS TO CONTROLS
1921					
May 25	19.0	135	148		
		200	220	1:425,000	1.48
May 28	21.0	120	114		
		165	157	1:425,000	1.38
May 20	20.0	218	218		
		218	218	1:450,000	1.00
May 21	20.0	143	143		
		148	148	1:450,000	1.03
April 27	22.5	115	101		
		107	94	1:500,000	0.93
April 29	20.5	55	54		
		55	4	1:500,000	1.00

Horizontal columns for which no strengths of adrenalin are indicated are the controls.

DISCUSSION

We consider that these experiments confirm the view of Boothby and Sandiford, cited in our opening paragraph, that adrenalin exerts a specific dynamic action on individual body cells. Since in none of our tests did the muscle make any movements whatever, the augmentation is of resting or basic metabolism as distinct from the metabolism of function.

Further experimentation is obviously necessary to determine whether this augmentation of metabolism is confined to muscular tissue or affects other tissues as well. With respect to the biological significance of the phenomenon not much can be said at present, but we feel justified in calling attention to certain findings of Gruber (7) relative to effects of adrenalin in counteracting the rise in stimulation threshold of muscles accompanying fatigue or curare poisoning. It will be recalled that Gruber showed adrenalin to be surprisingly efficient in restoring the normal low threshold of stimulation in mammalian preparations in which the threshold had been raised by repeated stimulation or by curare. Our finding that adrenalin augments the resting metabolism of muscle suggests the possibility that the effect of the hormone on stimulation thresholds may be an indirect one, depending on an im-

provement of conditions within the tissue brought on through the augmented metabolism. Until we can obtain more definite knowledge as to the relation of resting to functional metabolism in such tissue as muscle it will scarcely be profitable to pursue this suggestion further.

SUMMARY

1. By means of the indicator method of Haas the acid production in frogs' excised sartorius muscles during the half-hour following excision was found to average, during the spring months, 110×10^{-8}, expressed as C_H at 20°C.

2. Sartorius muscles immersed in solutions of adrenalin in Ringer's fluid showed definite augmentation of resting metabolism ranging from a 25 per cent increase at dilutions of 1:425,000 up to a fourfold increase at 1:20,000.

3. These results are interpreted as confirming the view of Boothby and Sandiford that adrenalin exerts a specific dynamic action on individual body cells.

BIBLIOGRAPHY

(1) Boothby and Sandiford: This Journal, 1920, li, 200; also 1921, lv, 293; also Sandiford, ibid., 1920, li, 407.
(2) Aub, Foreman and Bright: This Journal, 1921, lv, 293.
(3) Hill: Journ. Physiol., 1912, xliv, 466.
(4) Fletcher: Journ. Physiol., 1898, xxiii, 10; also 1902, xxviii, 354.
(5) Eddy and Downs: This Journal, 1921, lvi, 188.
(6) Haas: Science, N. S., 1916, xliv, 105.
(7) Gruber: This Journal, 1914, xxxiii, 335; also 1914, xxxiv, 89.

STUDIES OF CEREBRAL FUNCTION IN LEARNING

IV. Vicarious Function after Destruction of the Visual Areas

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The development of objective psychology has been due largely to the extension of the concept of reflex mechanisms from spinal reactions to include all of the more complex types of behavior by which the individual makes adjustments to his environment. In the hands of Bechterew, Pawlow, Kostyleff, Watson and others the conditioned-reflex has become the unit of behavior in all learned activities and explanation of even the most complex behavior resolves itself into the tracing of conditioned-reflex arcs through the central nervous system, together with analysis of the factors which determine their activity. Thus the mechanism of the conditioned reflex and, especially, the manner of formation of new arcs become one of the central problems of psychology.

Of details here we have almost no knowledge. The facts of cerebral localization tell a little of the paths of neural impulses but, when considered in the light of the reflex concept, much of current interpretation of the cerebral localization of "mental" functions becomes al nost meaningless and little is left save the fact that conditioned-reflex arcs traverse different parts of the cortex, the localization apparently depending upon the sense organs stimulated and the position of their projection fibers in the cortex. Beyond the sensory projection areas the arcs have not been traced. They do not necessarily descend through the pyramidal tract (12) and probably lie altogether outside of the so-called motor areas (8). Even the fundamental importance of the localized sensory projection areas seems questionable in view of the ease with which sensory function may be recovered after destruction of a normal sensory area. In lower mammals the destruction of any given third of the cerebral cortex does not abolish any sort of learning function and even in man the limits of recovery from cerebral injury seem determined only by reëducative methods.

This capacity of the nervous system for vicarious functioning in learning presents some of the most puzzling questions in the whole problem of the physiology of learning. The experiments of Treves and Aggozzotti (13), which seem to prove the possibility of learning in the pigeon after complete decerebration, show that the capacity for vicarious function is almost unlimited and much recent work goes to show that even in primates there is ability to compensate for the loss of large portions of the cerebral cortex. The partial destruction of a functional area produces a retardation in the performance of all of its functions, rather than a loss of specific elements, and even this retardation may disappear temporarily under conditions which lead to a general increase of tonus. Such facts argue for an almost equipotentiality of all parts of the central nervous system in the acquisition of new activities and present enormous difficulties for any simple account of the nervous mechanisms of learning.

Yet these same difficulties perhaps offer a point of attack upon one of the most important problems of the physiology of learning; that of the factors determining the course followed by the neural impulses in the performance of learned activities and of the laws which control the anatomical localization in the fixation of new conditioned-reflex arcs. The destruction of a functional area, followed by the reacquisition of that function by other parts of the nervous system, seems to present the problem of the fixation of arcs in habit in a more approachable form than does normal learning. The hereditarily defined paths are in part eliminated and the course of the conditioned-reflex arc must depend, to a greater extent than under normal conditions, upon the most fundamental and general relations of reflex paths to one another. The identification of areas functioning vicariously may make possible the working out of the anatomical relationships which have led to the activity of those areas and so throw light upon the normal conditions which determine the association of specific nervous elements in learning.

The existing data upon cerebral areas functioning vicariously are incomplete and conflicting. For the motor area the early investigators assumed that recovery from hemiparesis following unilateral destruction was due to the taking over of the lost function by the motor area of the opposite side. Carville and Duret (1) in 1875 proved that this is not true for the dog. Rothmann (12) has since obtained learning in monkeys after destruction of one motor area and of the pyramidal and rubrospinal paths of the opposite side, showing that in primates

the motor areas are not essential for recovery of motor function, and Franz and Oden (5) have found that even when intact the motor area of one side does not assume the function of the other when it is destroyed. I have shown that in the rat the cerebral motor nuclei (corpora striata) do not function vicariously for the stimulable cortex in the performance of learned activities, although there is some evidence that the two structures have an alternative dynamic function (8). Leyton and Sherrington (10) extirpated the thumb area in the gorilla and explored the adjacent parts of the stimulable cortex after the paralytic symptoms had disappeared, but failed to locate the part which was acting vicariously. Thus, such evidence as there is does not give any indication of the structures to which recovery from cerebral paralysis is due. A systematic exploration of the entire cerebrum after destruction of the motor areas has, however, never been made.

Studies of sensory and association areas have given more positive results, yet these are open to various interpretations and give little indication of the conditions which determine localization in vicarious function. Franz (2) destroyed the frontal lobes in cats and abolished a latch-box habit. The animals relearned the habit and a second operation on the area just back of the first lesion resulted in a second loss of the habit. It is possible that this is not a case of true vicarious functioning, but only of the assumption, by a part of a functional area, of the function of the whole area. Thus Franz and the writer (4), (9) found that partial destruction of the frontal region in the rat might interfere with the performance of somaesthetic-motor habits but that the whole area must be destroyed before the habits were completely abolished. It seems possible that the area destroyed by Franz in the second operation was one to some extent functional in the original acquisition of the habit, since his experiments do not show that destruction of this area in the intact animal does not interfere with the habit.

Vitzou (14) claimed that the visual function of one occipital lobe was assumed by the other after destruction of the first, that the destruction of the second lobe resulted in total blindness, and that the sectioning of the corpus callosum and one occipital lobe prevented the recovery of vision in the blinded eye. In view of later data upon recovery of vision after destruction of both occipital lobes in monkeys (3), his conclusions must be considered questionable.

None of these studies has been carried far enough to give an account of the mechanism of vicarious function or to suggest the anatomical

conditions for it. The motor cortex is not well suited for such investigations for its function is paralleled, especially in lower forms, by that of the corpus striatum (11), (15), (8); the area itself is probably more concerned with reflex tonus than with conditioned-reflexes, and in forms below the primates it is overlapped by the somaesthetic projection area.

The visual area in the rat offers greater advantages for an investigation of the problem. It is definitely restricted to the occipital pole and is the only part of the cerebrum concerned with the performance of visual habits. Destruction of it is invariably followed by loss of visual habits, yet closely adjacent areas may be destroyed without affecting visual discrimination. The visual habits, lost after its destruction, may be reacquired rapidly and are more easily recognized than latch-box habits. The question of possible reduction of the habits to subcortical levels by long training is eliminated, since I have shown that over-training to 1200 trials does not affect cerebral localization (7).

In the following experiments I have destroyed the visual areas in a number of animals, trained them after operation in discrimination of brightness and, by a second operation, explored the remaining parts of the cortex in an effort to discover what part of the cerebrum had assumed the visual function.

METHODS: *Operative technique.* For the first operation the skull was opened on each side, about 4 mm. in front of the parieto-occipital ridge. A small electric cautery was inserted cold and passed diagonally backward and downward through the cortex until the edge of the tentorium was felt. It was then heated until the tissue began to boil and drawn slowly back and forth so as to sever the entire occipital pole from the underlying structures. When hemorrhage was stopped the scalp wound was closed with interrupted sutures and covered with a cotton-collodion dressing. In the second operation essentially the same technique was used, but the lesions were made in the frontal, parietal, orbital or temporal regions.

Training. When the animals had recovered from the effects of the operation (usually after 10 days) they were trained to choose the illuminated alley and to avoid the darkened in a Yerkes discrimination box. Training was continued until they made 30 consecutive trials without error. In the following reports of cases the number of trials required for learning preceding the 30 errorless ones is given. Then follows a record of the number of trials in each day's training in which errors were made. Ten trials were given each day during training. Thus no. 26 (page 49) required 90 trials before 3 days without error

occurred. Following this the record shows that on the first day 6 of the 10 trials contained errors; on the second day, 5; on the third day 5; etc.

Tests of retention. After the animal had acquired the visual habit some part of the intact cortex was destroyed and retention of the habit following operation was tested. The retention tests were begun as soon after operation as the physical condition of the animal permitted, usually after 24 hours. Ten trials were given daily as in training and the numbers of errors and the general behavior of the animals recorded. The retention tests are thus retraining tests, and significant evidence of retention consists in the reacquirement of the habit in markedly fewer trials than were required for the initial learning.[1]

Methods of reconstruction. At the end of the retention tests the brains were removed and fixed in 10 per cent formalin and serial frontal sections, stained with iron hematoxylin, were prepared. Camera drawings of sections at intervals of ½ mm. throughout the length of the lesions were made and the exact extent of the lesions marked on them. The sections were referred to a diagram of the brain with projection of the important structures on the surface and their exact position determined. The dimensions of the lesions were then transferred from the drawings to the diagram with proportional dividers, the points so obtained were connected by lines and the areas inked in. This method is accurate to within ½ mm. with the diagrams reduced to natural size.

Only obvious lesions, absorption of tissue or complete degeneration, were recorded. The lesions shown are, therefore, minimal and in many cases it is certain that the actual injury was much greater. This is not a serious objection to the experiments since the facts obtained are of such character that, if true for small lesions, they are equally true though the lesions be larger.

THE VISUAL AREA. The only area of the cerebral cortex of the rat which is functional in vision lies on the dorsal convexity of the occipital pole. Figure 1 is a composite diagram of the extent of the lesions in seven animals which lost the habit of brightness discrimination following operation in this region. Four of these animals had received

[1] Other evidence of retention is obtained from the reactions of the animals in choosing between the alleys. Its interpretation has been discussed in detail before (6). Rapid withdrawal from the darkened alley; hesitation, with turning of the head toward first one, then the other stimulus plate; greater speed of reaction in the illuminated than in the darkened alley are invariable precursors of discrimination and are recorded as "discriminatory reactions."

less than 200 trials in the training box, three had received more than 1300 trials each. The blackened area in the diagram shows the area destroyed in all seven of the animals. The dotted areas were left undestroyed in one or another of them and are probably not functional in vision.

I have shown (6) that destruction of the temporal, parietal, frontal and superior orbital regions of the rat's brain or the combined destruction of the stimulable cortex and caudate nucleus (8) does not abolish the visual habit, if the occipital pole remains intact. In the published experiments the inferior temporal and orbital regions were not covered. The two following cases show the same result for these areas.

Fig. 1. Composite diagram of the extent of cerebral injury in seven animals which lost the habit of brightness discrimination after operation. The solid black areas were destroyed in all, the dotted areas in one or more.

No. 26.[2] Adult male. Trained in visual discrimination. Ninety trials were required for learning, with errors in successive tens of trials distributed as follows: 6:5:5:4:5:4:5:2:1:0:0:0:1:0:0:1:0:1:0:0:0. The temporal and orbital areas were destroyed on both sides by passing a cold cautery, bent to fit the curvature of the skull, over them and heating it until the tissue boiled. The animal showed pronounced shock, but was tested for retention 24 hours after operation. On this day he retained the somaesthetic habits of the box, but gave no evidence of discrimination. He was somewhat stuporous on this and the following day, but thereafter showed perfect discrimination.

Postoperative retention tests; errors in sucessive tens of trials: 6:3:1:0:0:0.

Extent of lesions. (Plate I, fig. 26.) Right hemisphere: The lesion extends from the level of the anterior commissure caudad to the posterior limit of the hippocampal lobe, including practically all of the temporal and parietal cortex within these limits. Left hemisphere: The lesion begins at the level of the anterior limit of the forceps of the corpus callosum and extends over the dorsal and orbital surfaces to the level of the corpus mammillaris.

[2] For convenience of reference these animals are numbered in sequence with others reported in earlier studies of the visual area (6), (7), (8).

No. 27. Small female. Trained in visual discrimination. One hundred trials were required for learning, with errors in successive tens of trials distributed as follows: 4:5:5:3:1:3:1:0:0:1:0:0:0. The temporal areas were destroyed by cauterization. Retention was tested 24 hours after operation. For 3 days the rat showed serious disorganization, motor incoördination, lack of orientation in the experiment box, and difficulty in finding food. On these days numerous errors were made. On the fourth day these difficulties had cleared up and discrimination was also perfect. No further errors were made in later tests.

Post-operative retention tests; errors in successive tens of trials: 5:2:6:0:0:0.

Extent of lesions. (Plate I, fig. 27.) Right hemisphere: The lesion extends from the level of the knee of the corpus callosum to the level of the anterior margin of the inferior colliculus, including all of the orbital and temporal regions between these limits and extending into the parietal and ectorhinal areas. Left hemisphere: The lesion extends from the knee of the callosum to the level of the anterior margin of the superior colliculus, including the orbital and parietal surfaces, but not extending so far over the temporal and ectorhinal areas.

Fig. 2. Composite diagram of the total extent of lesions in animals which preserved the habit of brightness discrimination after operation.

These two animals show almost perfect retention of the visual habit after extensive destruction of the orbital and temporal regions of both hemispheres. Their average number of errors in learning was 29.5; the average number in retention tests was 11.5, including the trials

therefore, that the occipital area shown in figure 1 is the only part of the cerebrum which is normally concerned with the performance of the habit of visual discrimination.

Relearning of the habit after destruction of the occipital pole of the cerebrum must thus involve either a wholly subcortical learning, or the utilization of parts of the cerebrum which are not normally important for the performance of the habit, and which are probably not functional in it when the occipital pole is intact.

TESTS FOR LOCALIZATION OF VISUAL HABITS FORMED AFTER DESTRUCTION OF THE VISUAL AREA. The majority of experiments seeking to determine the areas functioning vicariously after cerebral lesions have been unsuccessful, but they have all included only a small part of the cerebral cortex in the exploration following recovery from the effects of the first lesion. In the hope of identifying the areas functioning vicariously after destruction of the visual cortex, I have carried through a series of experiments which include exploration of every accessible part of the cerebral cortex, following lesion to the occipital lobes.

The general plan of the experiments was the following. The occipital cortex was destroyed in a number of animals and as soon as they had recovered from operation they were trained in visual discrimination of light intensity, until they made 30 or more consecutive trials without error. They were then subjected to a second operation by which some part of the remaining cortex was destroyed. Retention of the habit was tested as soon as possible after this operation; usually within 48 hours. The retraining of the retention tests was continued until the animal again gave clear evidence of discrimination.

For exploration of the cortex after occipital injury the animals were divided into four groups. In the first group the frontal pole, including about one-fourth of the intact area, was destroyed; in a second group

No. 27. Small female. Trained in visual discrimination. One hundred trials were required for learning, with errors in successive tens of trials distributed as follows: 4:5:5:3:1:3:1:0:0:1:0:0:0. The temporal areas were destroyed by cauterization. Retention was tested 24 hours after operation. For 3 days the rat showed serious disorganization, motor incoördination, lack of orientation in the experiment box, and difficulty in finding food. On these days numerous errors were made. On the fourth day these difficulties had cleared up and discrimination was also perfect. No further errors were made in later tests.

Post-operative retention tests; errors in successive tens of trials: 5:2:6:0:0:0.

Extent of lesions. (Plate I, fig. 27.) Right hemisphere: The lesion extends from the level of the knee of the corpus callosum to the level of the anterior margin of the inferior colliculus, including all of the orbital and temporal regions between these limits and extending into the parietal and ectorhinal areas. Left hemisphere: The lesion extends from the knee of the callosum to the level of the anterior margin of the superior colliculus, including the orbital and parietal surfaces, but not extending so far over the temporal and ectorhinal areas.

Fig. 2. Composite diagram of the total extent of lesions in animals which preserved the habit of brightness discrimination after operation.

These two animals show almost perfect retention of the visual habit after extensive destruction of the orbital and temporal regions of both hemispheres. Their average number of errors in learning was 29.5; the average number in retention tests was 11.5, including the trials made within 24 hours after operation, when both were suffering from shock. Both therefore gave certain evidence of retention following operation.

Figure 2 is a composite diagram of the lesions to the cerebrum of all the animals of this series (6), (7), (8) which have shown retention of the visual habit following operation. All of the cerebral cortex except the occipital lobes and the portions of the temporal lobes in contact with the floor of the cranial cavity have been covered by the operations, each of which included at least one-fourth of the total area indicated in the diagram. The habit has also been shown to survive the destruction of the motor area and both caudate nuclei (8). It seems clear.

therefore, that the occipital area shown in figure 1 is the only part of the cerebrum which is normally concerned with the performance of the habit of visual discrimination.

Relearning of the habit after destruction of the occipital pole of the cerebrum must thus involve either a wholly subcortical learning, or the utilization of parts of the cerebrum which are not normally important for the performance of the habit, and which are probably not functional in it when the occipital pole is intact.

TESTS FOR LOCALIZATION OF VISUAL HABITS FORMED AFTER DESTRUCTION OF THE VISUAL AREA. The majority of experiments seeking to determine the areas functioning vicariously after cerebral lesions have been unsuccessful, but they have all included only a small part of the cerebral cortex in the exploration following recovery from the effects of the first lesion. In the hope of identifying the areas functioning vicariously after destruction of the visual cortex, I have carried through a series of experiments which include exploration of every accessible part of the cerebral cortex, following lesion to the occipital lobes.

The general plan of the experiments was the following. The occipital cortex was destroyed in a number of animals and as soon as they had recovered from operation they were trained in visual discrimination of light intensity, until they made 30 or more consecutive trials without error. They were then subjected to a second operation by which some part of the remaining cortex was destroyed. Retention of the habit was tested as soon as possible after this operation; usually within 48 hours. The retraining of the retention tests was continued until the animal again gave clear evidence of discrimination.

For exploration of the cortex after occipital injury the animals were divided into four groups. In the first group the frontal pole, including about one-fourth of the intact area, was destroyed: in a second group the temporal and orbital regions were destroyed by lesions made continuous with the first operative field, along the sides of the brain: in a third group the parietal and superior temporal areas bordering upon the first lesion were injured: in the fourth group the initial lesion, produced before training, included both the occipital and parietal regions and after training the adjacent posterior frontal area was destroyed. Some animals with each combination of lesions showed retention of the habit and, as will appear, only those with accidental lesion to the thalamus lost it after the operation. The cases are therefore grouped in the following report with only the animals showing retention in the

first four groups and those showing loss in a separate fifth group. The records of the separate experiments follow.

Group I. Occipital: Frontal. The animals were trained after destruction of the occipital lobes. The frontal poles of both hemispheres were then destroyed and retention was tested.

No. 28. Adult male. In the first operation both occipital lobes were transected with a fine scalpel. Recovery was rapid and training was begun 5 days after operation. One hundred trials were required for learning with errors in successive tens of trials distributed as follows: 7:3:7:4:3:1:1:1:0:1:0:0:0. When the habit was established, the skull was trephined at the fronto-parietal suture, the holes enlarged forward, and the frontal poles of both hemispheres destroyed by incisions from the trephine openings to the base of the olfactory bulbs.

The first retention tests were made 4 hours after operation. The animal ran promptly, but spent most of the time exploring the problem box and after seven trials no longer went to the food. On the following day, 24 hours after operation, he made 95 per cent correct choices in 40 trials, giving certain evidence of retention. On later days he made no further errors.

Post-operative retention tests; errors in successive tens of trials: 5:0:1:1:0:0:0:0.

Extent of lesions. (Plate I, fig. 28.) First operation. Right hemisphere: The lesion begins on the dorsal surface above the hippocampus and extends caudad to the tip of the occipital pole, embracing all of the dorsal surface of the occipital lobe but leaving the lateral surface intact. There is a small lesion in the right superior colliculus. Left hemisphere: The lesion is similar to that on the right but broader in the anterior region and does not extend so far laterad on the occipital pole.

Second operation. On both hemispheres the lesion begins on the dorsal surface just back of the knee of the corpus callosum and extends diagonally forward to the tip of the frontal pole, destroying all of the cortex above the plane of the section.

The first operation destroyed all of the area striata in this animal. He then formed the habit of visual discrimination. After destruction of the entire dorsal surface of the frontal pole the habit was retained.

No. 29. Adult female. In the first operation the occipital lobes were transected. Training was begun five days after operation. Two hundred trials were required for learning, with errors in successive tens of trials distributed as follows: 5:5:2:5:4:7:6:2:2:1:1:1:2:2:0:2:0:1:3:1:0:0:0:0:0. The skull was trephined at the fronto-parietal suture and incisions extended from these openings to the base of the olfactory bulbs. The first retention tests were made 4 hours after operation. The animal showed excitement and gave few specific reactions to the problem-box situation. On the two following days her face was edematous and the eyes nearly closed. On the third day after operation the eyes were in better condition and clear evidence of discrimination was obtained. On the fourth and later days no errors were made.

Post-operative retention tests; errors in successive tens of trials: 1:2:0:0:0.

Extent of lesions. (Plate I, fig. 29.) First operation. Right hemisphere: The lesion begins at the level of the anterior limit of the hippocampal lobe and

extends caudad to the occipital pole, involving all of the cortex above the horizontal plane of the thalamus and all of the dorsal lobe of the hippocampus The lateral and ventral surfaces of the occipital lobe were not injured. There were a few slight injuries in the dorsal portion of the thalamus. Left hemisphere: The lesion begins at the level of the optic chiasma. One millimeter caudad to this it broadens to include all of the cortex of the dorsal surface to the tip of the occipital pole. The dorsal lobe of the hippocampus is destroyed.

Second operation. The lesion is identical in both hemispheres. It begins above the knee of the corpus callosum and extends diagonally forward to the frontal pole at the base of the olfactory bulbs. All of the cortex lying above the plane of this section is degenerated, and largely replaced by a cyst.

In this animal the first operation destroyed all of the visual area. She then formed the habit of visual discrimination and subsequent destruction of all of the frontal pole of both hemispheres did not abolish the habit.

These animals both showed practically perfect retention of the habit of visual discrimination after destruction of the frontal pole. The lesion included the so-called motor area for the head, face, neck and fore legs. Vicarious function in vision is not assumed wholly, if at all, by the frontal areas.

Group II. Occipital: Temporal. The animals were trained in visual discrimination after destruction of the occipital lobes. The orbital and temporal regions were then destroyed and the retention of the habit tested.

No. 30. Adult male. In the first operation the occipital region was destroyed by cautery. Training was begun 9 days after operation. One hundred sixty trials were required for learning, with errors in successive tens of trials distributed as follows: 8:6:5:7:3:4:4:4:2:3:1:1:1:3:1:1:0:0:0:0:0:0. The temporal lobes were next cauterized. Retention was tested on the second day after operation. The box-habits were perfect and only 1 error was made in 10 trials. On the following days certain evidence of retention was obtained.

Post-operative retention tests; errors in successive tens of trials: 1:1:1:0:0:0.

Extent of lesions. (Plate II, fig. 30.) First operation. Right hemisphere: The lesion extends from the level of the posterior commissure to the occipital pole with complete destruction of all of the cortex of the dorsal surface. Left hemisphere: The lesion is similar to that on the right but leaves the cortex of the postero-medial part of the occipital pole possibly functional.

Second operation. Right hemisphere: The lesion extends forward from the first injury to the level of the knee of the corpus callosum. Laterad it invades the orbital and temporal areas, destroying most of the inferior temporal lobe. Left hemisphere: The lesion is similar to that on the right but does not extend so far laterad.

The caudate nucleus, the anterior thalamic nucleus, and the superior geniculate body on the right are superficially injured.

After destruction of the temporal and part of the orbital surfaces, this animal showed retention of the visual habit.

No. 31. Adult male. In the first operation the parietal and occipital lobes were destroyed by cautery. Training was begun 9 days after operation. One hundred ninety trials were required for learning, with errors in successive tens of trials distributed as follows: 9:6:6:4:3:7:5:4:4:1:2:1:1:1:0:1:1:0:1:0:0:0:0:0. The trephine holes were enlarged forward and the cautery passed along the orbital and temporal surfaces. On the day following operation the animal was active and ran the box promptly, but gave no sign of discrimination. For the next 5 days he was stuporous and when placed in the discrimination box, wandered about slowly and failed to find the food. On the seventh day ten trials were obtained but he was still stuporous and disoriented. On the eighth day he was more active, made only three errors and gave evidence of reactions to the light. On the ninth day he was again stuporous. On the tenth day he seemed fully recovered and thereafter gave clear evidence of discrimination.

Post-operative retention tests; errors in successive tens of trials. (The day on which the record was made is indicated in parentheses until the tenth, after which each numeral represents one day's practice.) (1)7: (7)5: (8)3: (10)3: 2:0:0:0:0.

Extent of lesions. (Plate II, fig. 31.) First operation. Right hemisphere: The lesion extends from the level of the nucleus habenulae to the occipital pole, with destruction of all the cortex of the dorsal surface except a band along the postero-medial surface. Left hemisphere: The lesion is almost identical with that on the right.

Second operation. Right hemisphere: The lesion extends forward from the first injury to the level of the forceps of the corpus callosum, including almost all of the cortex of the orbital and temporal surfaces. A narrow band along the longitudinal fissure remains intact. Left hemisphere: The lesion is similar to that on the right but does not extend so far over the posterior temporal region.

This animal showed general deterioration following destruction of the orbital and temporal regions. The somaesthetic-motor habits of the problem box were lost. After ten days the general disorganization cleared up and the visual habit appeared. In spite of the severe operative shock, the habit was retained.

No. 32. Small adult female. The occipital lobes were cauterized in the first operation. Training was begun 10 days after operation. Eighty trials were required for learning, with errors in successive tens of trials distributed as follows: 7:6:3:6:5:5:2:3:0:0:0:0. The trephine holes were enlarged forward and the cautery passed over the anterior part of the first lesion and into the uninjured part of the temporal regions. Retention was tested 24 hours after operation. The animal made 4 errors in 10 trials but gave secondary evidence of retention in a few definitely negative reactions to the dark alley. On the second and later days she showed unmistakable evidence of retention, although some errors were made for many days.

Post-operative retention tests; errors in successive tens of trials:4:2:2:2:0:1: 2:3:2:3:0:0.

Extent of lesions. (Plate II, fig. 32.) First operation. Right hemisphere: The lesion begins at the level of the anterior margin of the superior colliculus and extends caudad to the occipital pole, including all of the cortex of the dorsal surface above the plane of the thalamus. Left hemisphere: The lesion begins at the same level as on the right, but fails to reach the extreme pole. A space equal

to the thickness of the cortex on the occipital pole remains intact, although its subcortical fibers seem to be injured and its capacity to function is doubtful.

Second operation. Right hemisphere: The lesion extends forward from the first to the level of the anterior margin of the hippocampus. Laterad it includes much of the cortex of the temporal region but leaves the inferior occipito-temporal region intact. Left hemisphere: The lesion is similar to that on the right, but covers more of the temporal region.

After destruction of a large part of the temporal region of both sides this animal showed little general disorganization. Numerous errors were made in visual discrimination, yet rarely more than 2 in any 10 trials after the first day. This small percentage of errors forms almost certain evidence of retention of the habit.

The animals of this group all show retention of the habit after more or less extensive destruction of the temporal and orbital areas. These regions alone have therefore not assumed the visual function.

Group III. Occipital: Parietal. The animals were trained after destruction of the occipital lobes. The parietal areas adjacent to the first lesion were then destroyed and retention tested.

No. 33. Adult female. In the first operation the occipital lobes were sectioned with a fine scalpel. Two hundred trials were required for learning, with errors in successive tens of trials distributed as follows: 2:6:6:1:8:6:5:4:6:4:2:1:2:0:0: 1:2:2:2:1:0:0:0. The trephine openings were enlarged forward for 3 mm. and a cautery passed laterad and mediad over the dorsal surface from these openings. Retention was tested 24 hours after operation. The animal showed some hesitation and excitement, but made few errors.

Post-operative retention tests; errors in successive tens of trials: 1:1:0:0:0:0.

Extent of lesions. (Plate II, fig. 33.) First operation. Right hemisphere: The lesion begins at the level of the anterior margin of the superior colliculus and extends caudad to include all of the cortex of the dorsal convexity of the occipital pole. Left hemisphere: The lesion begins at the same level as that on the right and includes all of the dorsal and lateral surfaces of the occipital region. There are extensive injuries to both hippocampal lobes.

Second operation: The lesions to both hemispheres extend cephalad from the first injury to the level of the anterior commissure and include all of the cortex of the parietal region.

This animal showed perfect retention of the visual habit after destruction of the parietal cortex.

No. 34. Adult female. In the first operation the occipital lobes were cauterized and the cautery was passed ventrad over the temporal lobes. Training was begun 10 days after operation. One hundred sixty trials were required for learning, with errors in successive tens of trials distributed as follows: 9:10:8:8:7:5: 5:5:2:3:1:0:1:1:1:2:0:0:0. The trephine holes were enlarged forward and the median parietal regions cauterized. Retention was tested 48 hours after operation. On the first day only 1 error was made in 30 trials, with clear secondary evidence of retention.

Post-operative retention tests; errors in successive tens of trials: 0:1:0:0:0.

Extent of lesions. (Plate III, fig. 34.) First operation. Right hemisphere: The lesion begins at the level of the commissure habenulae and extends to the occipital pole, including all of the cortex of the dorsal convexity and extending laterad to the inferior temporal area. Left hemisphere: The lesion is similar to that on the right, but does not include so much of the temporal region.

Second operation. The lesion is continuous with the first and includes all of the cortex of the dorsal convexity to the level of the knee of the corpus callosum on both hemispheres.

This animal showed perfect retention of the visual habit after complete destruction of the parietal area, after a preliminary operation which involved both the visual area and the greater part of the temporal regions.

No. 35. Adult female. In the first operation the occipital lobes were transected with a fine scalpel. Training was begun 8 days after operation. One hundred forty trials were required for learning, with errors in successive tens of trials distributed as follows: 5:7:6:3:5:3:4:6:5:5:4:4:4:4:0:0:0:1:0:0:0. The trephine holes were then opened and the dorso-median parietal region cauterized. Retention was first tested 18 hours after operation with no evidence of discrimination. The next tests were made 48 hours after operation and retention was then perfect. Post-operative retention tests; errors in successive tens of trials: 6:0:1:0:1:0:0.

Extent of lesions. (Plate III, fig. 35.) First operation. Right hemisphere: The lesion involves all of the occipital pole caudad to the anterior margin of the superior colliculus. The temporal lobe is uninjured. Left hemisphere: The lesion is identical with that on the right.

Second operation. Right hemisphere: The lesion extends from the anterior margin of the first injury to the level of the anterior commissure. All of the cortex of the dorsal convexity within these limits is degenerated. Left hemisphere: The lesion is similar to that on the right, but does not extend quite so far forward.

Destruction of the median parietal region in this animal did not abolish the habit formed after destruction of the visual area.

No. 36. Adult male. In the first operation the occipital lobes were destroyed by cauterization. Training was begun 7 days after operation. One hundred trials were required for learning, with errors in successive tens of trials distributed as follows: 5:4:6:6:3:4:0:1:2:2:0:0:0:0:0:0:0:0:0:0. The trephine holes were then opened and incisions made in the median parietal region with a scalpel. Retention was tested 4 hours after operation with no evidence of discrimination. On the following day this animal reacted to the problem box and sometimes seemed to give definite reactions to the dark alley, but made 4 errors. On the third and later days there was clear evidence of discrimination.

Post-operative retention tests, errors in successive tens of trials: 5:4:1:1:1: 1:0:0.

Extent of lesions. (Plate III, fig. 36.) First operation. Right hemisphere: The lesion begins at the level of the splenium as a median band which broadens caudad to include all of the cortex of the dorsal surface of the occipital pole. There is a superficial injury to the right superior colliculus. Left hemisphere: The lesion is similar to that on the right, but does not extend onto the temporal area.

Second operation. Right hemisphere: The lesion extends from the first to the level of the chiasma. In this area all of the cortex above the corpus callosum is destroyed. Left hemisphere: The lesion extends from the first to the anterior margin of the hippocampus.

Injury to the median parietal region did not abolish the visual habit in this animal.

These four animals show clear evidence of retention following destruction of the parietal areas adjacent to the first occipital injury. The parts in anatomical proximity to the visual area are therefore not solely responsible for the vicarious function.

Group IV. Occipito-parietal: Frontal. In order to assure that the first operation thoroughly destroyed the visual area and that the recovery of function was not due to small portions of its edge which escaped injury, a fourth group of animals was introduced in which the first operation was made very extensive and thorough. Large trephine holes were made in the parietal regions and the cautery or scalpel was passed along the external capsule back to the cerebellum and downward to the floor of the cranial cavity, so that the entire occipital fourth of the cortex was certainly destroyed. The second operation was made on the area adjacent to the first lesion.

No. 37. Adult male. In the first operation the occipital region and a part of the parietal were destroyed with a scalpel. Training was begun 8 days after operation. One hundred thirty trials were required for learning, with errors in successive tens of trials distributed as follows: 7:7:5:3:4:5:4:2:4:4:1:1:2: 0:0:0:0:2:0:0:1:0. The old trephine holes were opened and the remaining parietal area cauterized. Eighteen hours after operation the animal gave evidence of retention and 6 hours later made 30 consecutive trials without error.

Post-operative retention tests; errors in successive tens of trials: 1:0:0:0:0.

Extent of lesions. (Plate III, fig. 37.) First operation. Right hemisphere: The lesion begins above the anterior thalamic nucleus and includes all of the occipital pole. The cortex overlying the hippocampal lobe and colliculi is completely absorbed and that in the posterior temporal region (stippled in the figure) is degenerated. All of the cortex of the occipital and posterior temporal regions was thus non functional. Left hemisphere: The lesion is similar to that on the right but does not extend to the temporal region.

Second operation. Right hemisphere: The injury extends as a narrowing band from the first along the dorso-lateral surface of the cortex to the level of the genu. Left hemisphere: The lesion includes all of the cortex of the dorsal convexity from the first injury to the anterior margin of the forceps of the callosum.

The first operation in this animal destroyed the posterior part of the parietal area and the entire occipital pole. The visual habit was acquired after the operation and survived the subsequent destruction of the remaining parietal and the dorsal part of the frontal regions.

No. 38. Adult female. In the first operation the occipital lobes were cauterized. Training was begun 7 days after operation. One hundred ninety trials were required for learning, with errors in successive tens of trials distributed as follows: 8:5:4:4:3:1:0:1:2:0:1:1:0:1:1:1:0:0:1:0:0:0:0. The trephine holes were again opened and the cautery passed forward from them for about 3 mm. into the median parietal region. Retention was tested 24 hours after operation and conclusive evidence of discrimination obtained.

Post-operative retention tests, errors in successive tens of trials: 1:1:0:0:0.

Extent of lesions. (Plate IV, fig. 38.) First operation. The injuries produced by the first and second operations are continuous and their boundaries difficult to determine. The first probably begins, on both hemispheres, above the splenium and extends to the occipital pole. All the cortex dorsad to the hippocampus and the entire occipital pole is absorbed. There are slight superficial injuries to both superior colliculi.

Second operation. All of the dorsal convexity of both hemispheres up to the level of the anterior commissure is replaced by a cyst.

Cauterization of the median parietal region did not abolish the habit in this animal.

No. 39. Adult male. The occipital lobes were cauterized in the first operation. Training was begun 10 days later. One hundred seventy trials were required for learning, with errors in successive tens of trials distributed as follows: 7:6:4:3:3:4:2:1:2:0:1:1:1:0:1:0:0:0:0:0. The trephine holes were reopened and the cautery passed into the median parietal region for about 3 mm. in front of the first lesion. Retention was first tested 14 hours after operation. The animal was slow and stuporous for 3 days and gave little evidence of retention. This condition improved gradually and discrimination appeared on the fourth day. Some disurbance persisted, however, and on the 7th, 8th, 11th, 14th and 15th days his reactions were so slow that no tests could be made. Somewhat less practice was required for relearning than for the original learning.

Post-operative retention tests, errors in successive tens of trials: 4:7:4:1:2:3: 0:0:2:2:1:0:0.

Extent of lesions. (Plate IV, fig. 39.) The lesions made by the first and second operations are continuous and it is difficult to determine the line between them. The interpretation of the case depends upon whether the injuries to the thalamus were inflicted in the first or second operation. The slow recovery after the second operation makes it probable that at least a part of the damage to the subcortical structures was done then, yet the survival of the habit does not support this view. It is possible that some injury to the thalamus occurred in both operations. The following interpretation of the lesion seems the most probable from the anatomical evidence, but is not certain.

First operation. Right hemisphere: The lesion begins at the level of the anterior thalamic nucleus and extends caudad to include all the cortex behind this level except the inferior temporal region. The inferior temporal area is reduced in thickness and its lower layers are partly degenerated as a result of the passage of the cautery along the external capsule. Left hemisphere: The injury is similar to that on the right, but the complete degeneration does not extend to the temporal area. The cortex in this region is unusually thin, however, and is probably non-functional.

Second operation. Right hemisphere: The lesion extends from the first to the level of the anterior commissure, including all of the cortex of the dorsal convexity within these limits. Left hemisphere: The lesion is similar to that on the right but does not extend so far cephalad.

There are extensive injuries to the optic radiations and superficial degeneration of the external geniculate bodies of both sides. Both superior colliculi are injured, that on the left most extensively.

Destruction of the median parietal area with possible injury to the thalamus in this animal resulted in some disturbance but not a complete loss of the visual habit.

These animals all gave evidence of retention after the second operation. In no. 39 the results were somewhat obscured by operative shock, but a comparison of the errors in learning and retention tests indicates that some discriminative ability survived the operation and the injury to the thalamus doubtless accounts for the partial loss. In the case of the other two animals the evidence for retention is unquestionable.

Group V. Cases showing loss of the habit. Only two animals out of the entire number studied failed to give evidence of retention following the second operation. Their records follow.

No. 40. Adult male. In the first operation the occipital lobes were deeply incised with a scalpel. Recovery was slow and training was begun 18 days after operation. One hundred twenty trials were required for learning, with errors in successive tens of trials distributed as follows: 10:5:5:1:3:2:0:0:1:0:1:0:0:0:0. The trephine holes were enlarged forward and the knife passed forward and laterad in an effort to destroy the parietal and temporal regions. For 2 days the animal showed marked disturbance, rotating sharply to the right, failing to react to the problem box, and having difficulty in finding food. On the third day the somaesthetic-motor habits of the problem box reappeared but there was no clear evidence of discrimination for 130 trials. Training was continued until the problem was relearned, which required 210 trials.

Post-operative retention tests; errors in successive tens of trials: 3:5:5:5:5:6:4:2:2:3:2:5:3:1:3:1:1:2:3:1:1:0:0:0.

Extent of lesions. (Plate VI, fig. 40.) First operation. The lesion begins above the hippocampal lobe at the level of the thalamo-mammillary tract and extends caudad to the occipital pole. All of the cortex overlying the hippocampus except the inferior temporal region is destroyed. Left hemisphere: The lesion is similar to that on the right but does not involve the superior temporal region.

Second operation. Right hemisphere: The lesion is continuous with the first and extends cephalad to the level of the knee of the corpus callosum. All of the cortex of the dorsal convexity within these limits is replaced by a cyst. A part of the orbital surface is involved. Left hemisphere: The lesion is similar to that on the right but extends almost to the frontal pole and does not reach the orbital surface.

There are extensive injuries to subcortical structures. The dorsal lobes of both hippocampi were injured by the first operation which also destroyed parts of both superior colliculi. The second operation resulted in slight injuries to the

optic radiations and external geniculate bodies and rather extensive injuries to the anterior thalamic nuclei. The right caudate nucleus was severely injured.

This animal formed the visual habit after destruction of the occipital pole and lost it again after extensive injuries to the frontal and parietal cortex and to the subcortical optic nuclei. The loss of the habit was almost certainly due to the subcortical lesions, since the habit has survived destruction of corresponding cortical areas in other animals. The habit was relearned after the second operation, but with difficulty.

No. 41. Adult male. In the first operation the occipital lobes were destroyed with the scalpel. Training was begun 8 days after operation. One hundred forty trials were required for learning, with errors in successive tens of trials distributed as follows: 4:6:6:6:3:2:0:4:3:1:1:0:0:1:0:0:0:1:0:1:0:1:0. The temporal region was cauterized. Eighteen hours after operation the somaesthetic habits of the problem box were retained, but there was no evidence of visual discrimination, and retention tests on later days gave no indication of retention. Training was continued until the habit was reëstablished, after 210 trials.

Post-operative retention tests, errors in successive tens of trials: 3:3:5:5:4:4 3:3:8[3]:9:5:5:5:5:5:4:4:5:1:1:1:0:0:0.

Extent of lesions. (Plate IV, fig. 41.) First operation. Right hemisphere: The lesion begins at the level of the anterior thalamic nucleus and extends to the occipital pole, including all of the cortex of the dorsal surface, but leaving the temporal region intact. Left hemisphere: The lesion is similar to that on the right.

Second operation. Right hemisphere: The lesion is continuous with the first and extends cephalad to the level of the knee of the callosum. Laterad it includes all of the temporal region to the ectorhinal area. The right caudate nucleus is partially destroyed. There are extensive degenerations in the region of the external geniculate body and the anterior thalamic nucleus. Left hemisphere: The lesion is marked by a vast clot which extends along the external capsule from the level of the anterior commissure to the occipital pole. All of the cortex of the parietal and temporal areas, from the pillars of the fornix to the inferior colliculus is either destroyed by the cautery or so severely involved in the clot as to be certainly non-functional. The left caudate nucleus is slightly injured. The optic radiations are involved in the hemorrhage and seemingly completely destroyed. There is some degeneration, marked chiefly by enlargement of blood vessels, in the external geniculate body.

Following occipital operation this animal formed the visual habit. Destruction of the parietal and temporal regions and of thalamic nuclei resulted in the loss of the habit.

SUMMARY OF EXPERIMENTS. In the fourteen animals described above the occipital poles of both hemispheres were destroyed by an operation known to abolish visual habits in normal rats. The animals were then trained to perfect performance in the habit of discrimination of light intensity. After training a second cortical area was destroyed

[3] The rise here was due to an infection of the scalp which made the animal ill for several days.

and retention of the habit tested after this operation. The four combinations of occipito-frontal, parietal, temporal and occipito-parietal with temporal covered all parts of the cerebral cortex except those in contact with the floor of the cranial cavity. Table 1 summarizes the data on learning and retention in these animals.

Rats 40 and 41 failed to give any evidence of retention of the habit and required a greater amount of practice for relearning than for the original learning. In both these animals the radiations of the optic

TABLE 1

The numbers of trials and errors required for learning the habit of visual discrimination in the absence of the visual cortex and for reacquiring it after a second operation, in animals described in this series. Each "error" represents a trial in which one or more mistakes was made.

GROUP	ANIMAL NUMBER	LEARNING		RETENTION	
		Trials	Errors	Trials	Errors
I. Occipitofrontal	28	100	28	30	2
	29	200	52	20	3
II. Occipitotemporal	30	160	54	30	3
	31	190	57	50	20
	32	80	37	100	21
III. Occipitoparietal	33	200	61	20	2
	34	160	68	20	1
	35	140	66	50	8
	36	100	33	60	13
IV. Occ.-par.-temporal	37	130	49	10	1
	38	190	34	20	2
	39	170	36	110	26
V. Thalamic	40	120	28	210	63
	41	140	37	210	88

nerve or the visual nuclei of the thalamus were severely injured. The injuries to the cortex were not essentially different from those of other animals which retained the habit (rats 31, 34, 35) so that it is quite certain that the loss of the habit in these cases was due to the interruption of the visual paths at subcortical levels.

One other animal (no. 32) required more trials in retention tests than in learning. Her relearning consumed 25 per cent more trials than learning, but 43 per cent fewer errors were made and her general

behavior in the problem box gave clear indication of discriminatory reactions after the first 10 trials of the retention tests. In two other cases the number of trials required for relearning exceeded 50 per cent of the number for learning (no. 36 and 39). Rat 36 made few errors, however, and gave evidence of retention by discriminatory reactions. Rat 39 received injuries to the thalamus which were almost certainly responsible for the retardation following operation. In all three cases the evidence from the numbers of errors made indicates a fair degree of retention. Discriminatory reactions in the retention tests support this interpretation and, finally, other animals with nearly identical lesions gave clear indication of retention so that these, at most, can only be considered cases of loss from operative shock.

In the remaining nine animals, which include cases with each type of operation, the evidence for retention of the habit is unquestionable. Rats 28, 29, 30, 33, 34, 35, 37 and 38 made an average of 51.5 errors in learning, as contrasted with 2.75 in retention tests. There is clear evidence that the habit survived every combination of cortical operations which was tested and that where certain loss followed the operation it was due to accidental injury to subcortical tracts.

In every case studied the first operation included all of the area destruction of which abolishes the habit in the normal animal. In the majority it involved the entire occipital pole back of the anterior margin of the hippocampus. Figure 3 shows the combined extent of the lesions to the occipital region resulting from the first operations in the 12 animals which gave evidence of retention of the habit after the second operation. The area destroyed in all the animals except Nos. 40 and 41 is indicated in solid black. There is no relation between the extent of destruction in the first lesion and the retention or loss of the habit after the second operation. In each group there was at least one animal with almost complete destruction of the entire occipital pole. It is certain therefore that the acquirement of the visual habit was not due to the activity of some part of the margin of the normal visual area which remained undestroyed by the first operation. The learning was due to the activity of some structure which does not have important visual function in the normal animal and must be considered a true case of vicarious function.

The total extent of all second operations after which the habit was retained is shown in figure 4 which is a composite diagram made by superimposing the diagrams of the second lesions in plates I to IV, figures 28 to 39 inclusive. This area includes all of the cerebral cortex

outside of the first lesions except the ectorhinal and inferior temporal regions. In no. 31, one ectorhinal area was destroyed and in nos. 31 and 37 the greater part of the inferior temporal regions was rendered non-functional.

Since the habit survived each combination of operations, it is clear that neither the frontal, parietal, orbital or superior temporal region

Fig. 3. Composite diagram of the lesions to the visual area in those animals of the present series which acquired the habit of visual discrimination after this operation and retained it after a second operation. The solid black area was destroyed in all. The stippled area was destroyed in one or more.

Fig. 4. Composite diagram of the extent of all second operations, following which the habit of visual discrimination was retained. The first operations in these animals comprised the area shown in figure 3.

assumes, alone, the function which is normally mediated by the occipital cortex, and it is very improbable that either the ectorhinal or inferior temporal areas or the caudate nuclei play any more important part in the vicarious functioning. The nearly complete exploration of the cerebrum has failed to reveal any specific area which assumes the visual function in the absence of the visual area.

THE NATURE OF THE VICARIOUS FUNCTION OF THE CEREBRUM. Three explanations of the above results are possible. First, the visual function

may have been assumed by the areas remaining unexplored by the second operation. These were only the left ectorhinal and the inferior temporal regions. The anatomical relations of these parts give no reason for assuming that they are more closely related to vision than the parts explored, and in normal animals the visual habit survives their destruction. The earlier study of the formation of visual habits (6) after destruction of various parts of the cortex did not cover these areas, but in the study of the learning of the "double-platform box," reported at the same time, it appeared that these areas could be dispensed with as readily as any other part of the cortex, and more recent work has indicated a visual element in that habit. There seems to be no reason for supposing that the destruction of these areas would have produced any different effect from those found in the exploration of other areas, and the small size of the areas and the fact that they were invaded and partially destroyed in several of the animals without loss of the habit makes it almost certain that these areas do not alone assume vicarious function in vision.

The second possibility is that the habit was learned wholly at subcortical levels. Data now being accumulated indicate that the destruction of large cerebral areas (more than 60 per cent of the cortex) greatly retards or abolishes the power to form visual habits and make this hypothesis improbable. I have made several attempts to test the survival of the habit after a second operation involving almost complete decerebration but have not obtained long enough survival to assure that the loss was not due to operative shock. One case has been obtained which bears upon the question, however. In an attempt to repeat the present experiments with a somaesthetic habit mediated by the frontal pole, animals were trained in a latch-box habit. One of these lost the habit after destruction of the frontal areas, relearned it, and then developed an infection of the cerebral substance. As the infection progressed the animal lost the habit again, but retained some of the simpler somaesthetic habits of the problem reaction, of the type which survives injury to any part of the cerebrum. Autopsy showed one hemisphere replaced by a cyst and all the dorsal and lateral convexities of the other destroyed. The thalamus and lower centers were intact, so far as could be determined by gross histological examination. The retention of the simpler habits in this animal indicated that the loss of the more complex was due to the lesions and not to toxic effects of the infection. The case seems to show that complete destruction of the cortex abolishes a vicarious function which may survive lesser

injuries, and that the cortex is the essential structure in this vicarious function. The evidence is, perhaps, not conclusive, but tends to exclude the possibility that the recovery of function is due solely to the activity of subcortical centers.

The third possibility is that of a diffuse functioning of the cortex, such that the various parts are equipotential in the habit. The destruction of the entire cortex seems to result in the loss of habits acquired by vicarious functioning and to limit the possibility of forming complex visual habits. The explorations were extensive enough to make it improbable that any localized area functioning in vision remained intact in all the animals. No other possibility remains, then, save that the area functioning vicariously overlapped the different operative fields so that enough of it survived each operation to retain the visual function. But the variations in the operations performed make it certain that no limited area could have remained in part undestroyed in all the operations. We must conclude, therefore, that in vicarious functioning all parts of the cerebral cortex (within undetermined limits of size) remaining intact are capable of mediating the habit.

Such a condition agrees with that obtaining among the separate parts of a limited functional area under normal conditions. Thus Franz (2) found that the latch-box habit in cats survived the destruction of the frontal pole of either hemisphere, although it was abolished by destruction of both. Lashley and Franz (9) verified this for the rat and showed that other partial destructions of the area give like results. In recent experiments I have found that the visual habit survives the destruction of either occipital lobe in combination with either eye, although the habit is lost after destruction of both lobes (decussation is apparently complete in the rat and there is no indication of a hemianopsia).

In such partial destructions of functional areas, when the lesion is extensive, there is a retardation or difficulty of performance of the habit as a whole, rather than a loss of specific parts of it. This indicates that the intact portion of the area contains representation of the entire habit but lacks the dynamic function of the whole area. Similar phenomena are observed in apraxia and aphasia in man and suggest that equipotentiality of the parts of functional areas is a constant condition in cerebral function.

Such an equipotentiality of function demands the existence of numerous fibers passing to and from all parts of the area, so that each conditioned reflex is mediated by numerous equivalent arcs, conducting

impulses which summate for the performance of the given act. With this mechanism, destruction of a part of the area might reduce the summated effects of the impulses without changing their specific character, and so lead to a difficulty of performance (need for tonic reinforcement) rather than a complete loss of any elements of the habit.

The data from the present experiments indicate that in the absence of a functional area, the same laws hold for the vicarious functioning of the entire remaining cortex as for the separate parts of the functional area when it is present. The function of the destroyed area is probably assumed by scattered fibers in all parts of the cortex, so distributed that the further destruction of any limited area does not involve a sufficient number of fibers to retard seriously the performance of the habit.

The experiments give no clue to the factors which determine localization under normal conditions. There is no localization of vicarious function in the absence of the normal area for the function, when a single habit is learned. The normal area is characterized chiefly by a concentration of projection fibers from specialized subcortical nuclei within its limits. It may be that normal localization is determined solely by this preponderance of specialized afferent fibers, yet this explanation does not seem in accord with the fact that other cortical areas can be just as efficient as the normally functional area in the performance of the habit.. It is possible that, when the reflex arcs of one habit are laid down, others tend to group around them as similar habits are formed, and thus to develop functional localization in the region of the dominant or most numerous arcs of the original habit. This possibility is now being tested by early destruction of the visual area and continued training in visual habits.

SUMMARY

The normal visual area of the cerebrum was destroyed in fourteen rats. The animals were then trained to discriminate differences of light intensity. When the habit was acquired a second operation destroyed some other cerebral area and tests were made to determine the loss or retention of the habit after this operation. The habit survived the destruction of any given third of the cortex left intact by the first operation. Only a small area in contact with the floor of the cranial cavity remained unexplored and it seems certain that there is no cerebral localization in vicarious functioning for the visual area.

BIBLIOGRAPHY

(1) CARVILLE ET DURET: Arch. d. Physiol., 1875, 2e Série, i, 352.
(2) FRANZ: Arch. Psychol., 1907, no. 2, 1.
(3) FRANZ: Psychol. Monogr., 1911, xiii, no. 4., 1.
(4) FRANZ AND LASHLEY: Psychobiol., 1917, i, 3.
(5) FRANZ AND ODEN: Psychobiol., 1917, i, 33.
(6) LASHLEY: Psychobiol., 1920, ii, 55.
(7) LASHLEY: Journ. Comp. Psychol., 1921. In press.
(8) LASHLEY: Brain, 1921. In press.
(9) LASHLEY AND FRANZ: Psychobiol., 1917, i, 71.
(10) LEYTON AND SHERRINGTON: Quart. Journ. Exper. Physiol., 1917, xi, 135.
(11) LUCIANI: Human physiology, London, 1915.
(12) ROTHMANN: Arch. f. Anat. u. Physiol. (Physiol. Abt.), 1907, 217.
(13) TREVES ET AGGOZZOTTI: Arch. ital. d. biol., 1901, xxxvi, 181.
(14) VITZOU: Journ. Physiol., 1898, Suppl., 57.
(15) WILSON: Brain, 1913, xxxvi, 427.

In the plates the figures are numbered to correspond to the serial numbers of the animals which they represent. In figures 28 to 41, the first lesion is always that indicated on the occipital pole. The second lesion appears in various positions and, where continuous with the first, it is separated from the latter by a dotted white line.

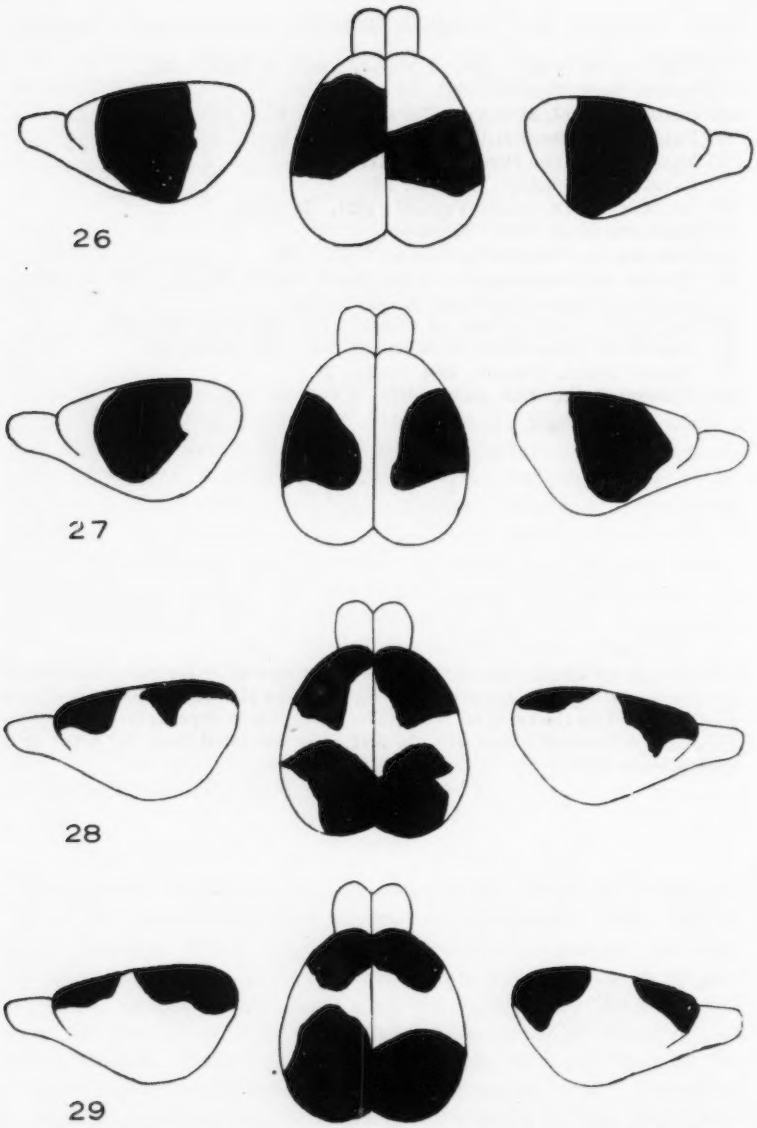

Plate I. Figs. 26–27. Extent of the lesions in orbital and temporal regions which failed to abolish visual discrimination.

Figs. 28–29. Destruction of the occipital pole, followed by training. Subsequent destruction of the frontal regions with survival of the habit. *Group 1.*

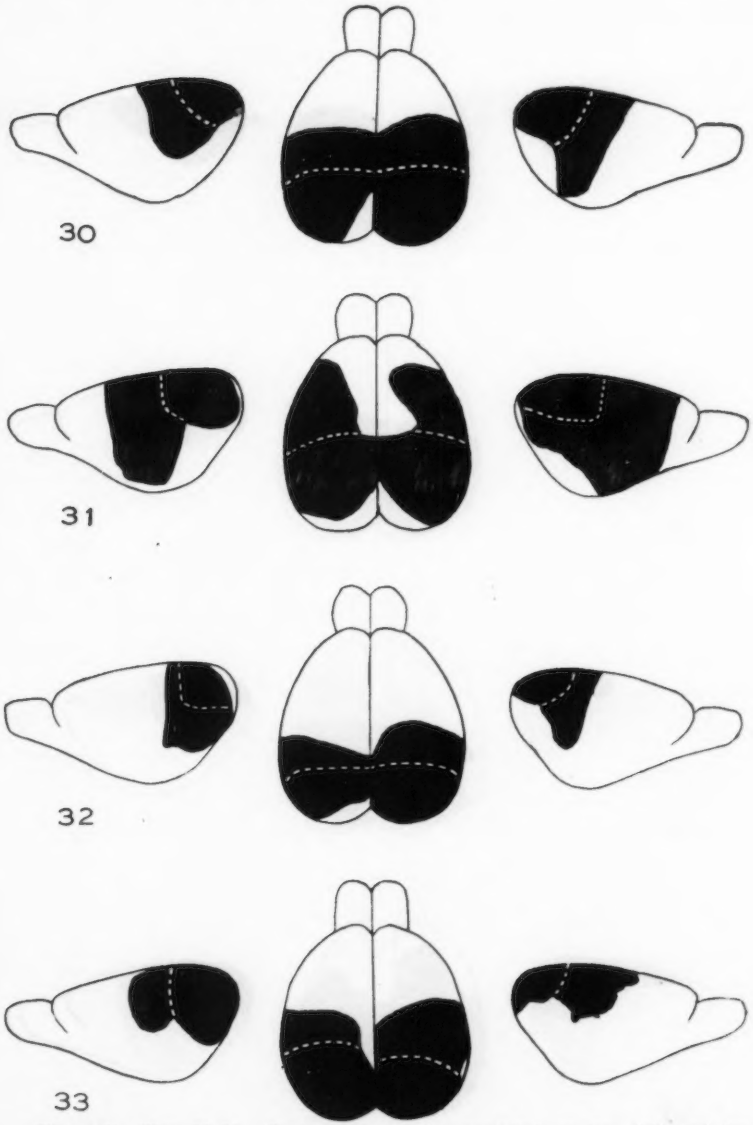

Plate II. Figs. 30–32. Destruction of the occipital region, followed by training. Subsequent destruction of the temporal and orbital areas with retention of the habit. *Group 2.*

Fig. 33. Destruction of occipital region followed by training. Subsequent destruction of parietal region with retention of the habit. *Group 3.*

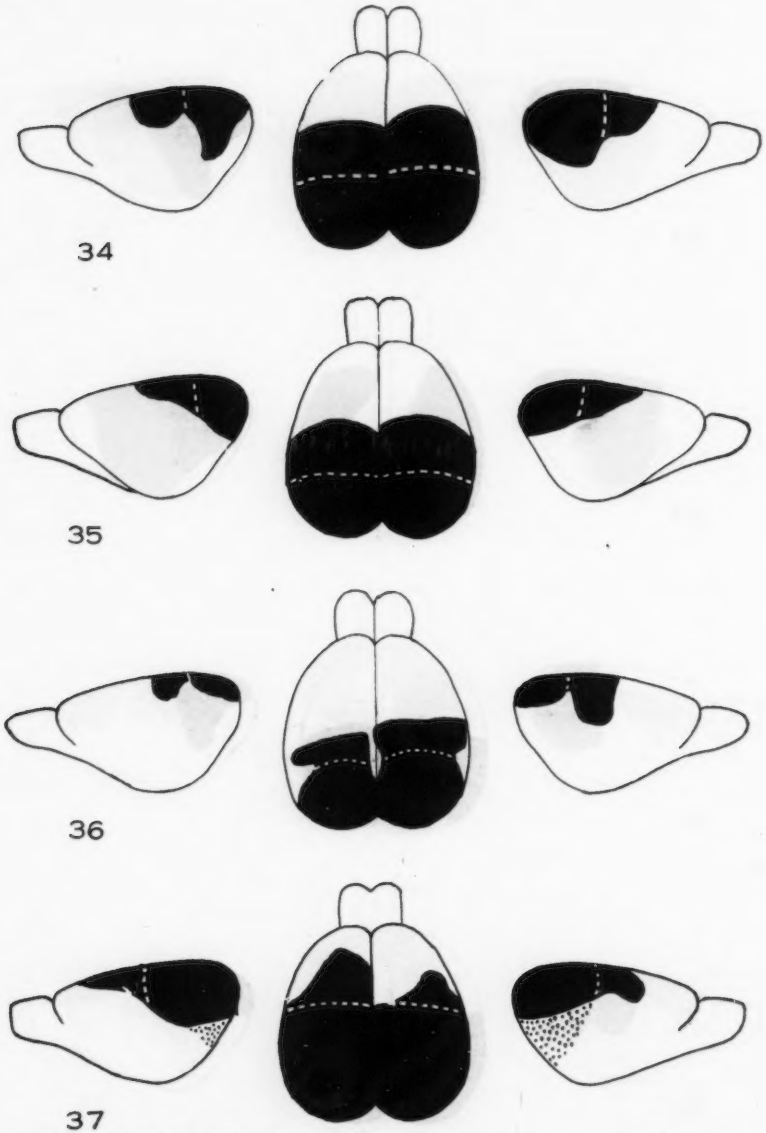

Plate III. Figs. 34–36. Same as figure 33.

Fig. 37. Destruction of post-parietal and occipital regions followed by training. Subsequent destruction of the posterior part of the frontal region with retention of the habit. *Group 4.*

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Plate IV. Figs. 38–39. Same as Fig. 37.

Figs. 40–41. Lesions in animals which lost the habit of visual discrimination after the second operation. *Group 5.*

OBSERVATIONS ON THE MOTILITY OF THE DUODENUM AND THE RELATION OF DUODENAL ACTIVITY TO THAT OF THE PARS PYLORICA

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In an earlier paper, in which the relation of motility in the antrum to motility in the pyloric sphincter was discussed, attention was called to the observation that certain alterations occurring in these parts were associated with duodenal activity (1). The experiments here reported bear specifically on the relation of duodenal motility to the motor activities of the stomach.

Methods. The observations presented herein are based on graphic observations made on 23 dogs. In addition to these experiments, direct observations were made upon 3 dogs the viscera of which had been exposed in a warm saline bath. Graphic records were obtained from both anesthetized and conscious animals at various intervals of time following the operation for the placement of apparatus. Ether anesthesia was used throughout operative procedures. In 3 dogs a morphine narcosis (10 mgm. per k. subcutaneously) was employed and ether used only during the actual preparatory operation.

Briefly, the operations consisted of placing the chambers of a compound enterograph (1) in the first portion of the duodenum and in the antrum or pyloric canal through an opening in the fundic portion of the stomach. After securing the apparatus in position the openings in the stomach and abdomen were closed separately about the small, heavy rubber tubes leading from the chambers (balloons) of the enterograph. Aseptic precautions were observed in all cases in which the animal was permitted to survive for a period longer than 12 hours. In two experiments a simple, rigid pylorograph, previously described (2), was placed in the lumen of the sphincter and in the first part of the duodenum a free balloon, the conduit from which was brought out through the walls of the duodenum and abdomen. This procedure was abandoned by

reason of the immobility of the duodenum resulting from the trauma incident to operative methods.

The various types of apparatus used in obtaining graphic records are modifications of the pylorograph (2) and double-chambered enterograph (1). These enterographs consist of chambers (modified balloons) which, when in position, occupy the first portion of the duodenum and either the lumen of the antrum or the pyloric sphincter. Triple-chambered enterographs also were constructed for the purpose of obtaining synchronous readings of the activities in the antrum, sphincter and first part of the duodenum. The details of construction of these devices are illustrated diagrammatically in figure 1. The chambers of the

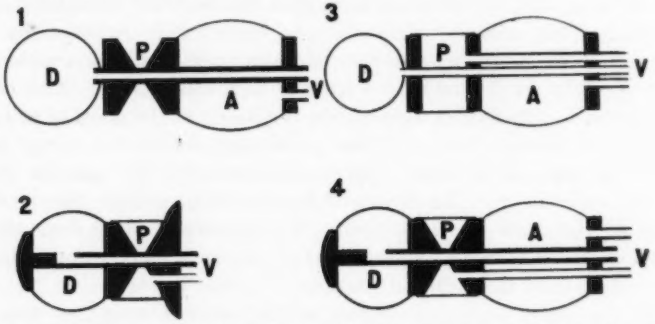

Fig. 1. Diagrams of various types of enterographs. *1*, Double-chambered, semi-rigid enterograph used in obtaining synchronous antral and duodental tracings. *P*, Sloping surface spool for sphincter fixation. Fixation was further secured by a ligature around the base of the spool stitched into the antral musculature. *2*, Rigid, double-chambered enterograph used in obtaining synchronous readings of sphincteric and ducdenal activity. The large flange on the gastric side of the sphincter chamber acts as a barrier to the passage of the apparatus into the duodenum. Fixation was further secured by stitching the ligatures about the bases of the sphincter chamber into the musculature of the gastric and duodenal walls. *3*, Flexible, triple-chambered enterograph used in obtaining synchronous readings of antral, sphincter and duodenal motility. The vents leading from the three chambers are heavy rubber tubes. The disadvantage of this device is that pressure on the distended chambers tends to lengthen their long diameter. *4*, Rigid, triple-chambered enterograph constructed so that pressure on the chambers can only result in the diminution of their cross diameter. Fixation of this apparatus is similar to that used for the double chambered enterograph.

D, Duodenal chamber; *P*, pyloric chamber; *A*, antral chamber. *V*, Vents connected by heavy rubber tubing to water manometers. Chambers *D* and *A* are covered with condom rubber; *P*, with a section of finger cot. Portions shown in solid black are made of hard rubber.

enterograph are connected by means of heavy-walled rubber tubing with manometers carrying from 5 to 30 mm. of water pressure for balloon distention. Piston, bellows and tambour recorders were used for the graphic registration of motility. Save for the water manometers, air transmission was used entirely.

Graphic records were obtained from stomachs recently emptied and from those containing various amounts of food; starved animals were not studied.

Results: Direct observations on the exposed duodenum. Motility in the duodenum, exposed in a warm saline solution, arises as a constriction band on the hepatic surface approximately 2 cm. distal to the pyloric ring, or in the terminal portion of the so-called "duodenal cap" in man. This primary contraction is immediately followed by the contraction of increasing numbers of fibers until finally a considerable portion of the duodenum is in a state of high contraction. This phase is followed by rapid relaxation which, beginning at the point of primary contraction, passes down over the previously constricted area. Such motor phenomena at times appear rhythmically, the positive phase of the cycle in the duodenum following immediately the positive phase of the cycle in the antrum. The duodenum may demonstrate a high degree of tonicity (contraction) and active segmentation from the distal portion of the "cap" to a point 2 inches distant.

The rhythmic segmental activity of the small intestine, so well described by Cannon (8), is readily discernible in the duodenum; however, there often appear in this region of the gut movements which do not conform to the segmental type of motility. Of this more will be said in the discussion of our graphic findings. A given portion of the duodenum often executes rhythmic contractions of equal strength (segmental contractions) for a considerable period of time. At other times the same portion of intestine may show groups of contractions, the first of which is powerful, followed by from one to four contractions of varying intensity, such a series of variations being similarly and successively repeated. At no time was segmental action seen in the "cap" region; however, this part of the intestine showed alterations in size which apparently depended upon the degree of force with which material was delivered into and withdrawn from it.

Graphic observations on duodenal activity. The most constant type of motility in the first part of the duodenum, as indicated by our graphic methods, is rhythmic segmentation (8). The segmental contractions occur at the rate of 3 to 5 per antral cycle, i.e., 15 to 25 a minute. An

individual segmental action, as has been shown by previous investigators, is characterized by a contraction and a relaxation phase

Graphic records obtained by us usually showed changes which corresponded to alterations in intra-abdominal pressure incident to respiration. Before proceeding further we wish to discuss briefly these changes both because they were unavoidably recorded and because in some instances at least they might be confused with the motor activity of the gut itself.

These respiratory markings are especially well defined or even exaggerated in cases where duodenal activity and tone are diminished. When, on the other hand, the duodenum is highly active, respiratory movements affect the recording apparatus only slightly and appear as negligible irregularities on the duodenal curves. The influence of these respiratory changes in an animal demonstrating reduced duodenal motility is shown graphically in figure 2. Here, during the completion of one sphincter cycle, *Y*, there occur eight acts of respiration superimposed upon what appear to be three and one-half feeble duodenal contractions. In contrast to this marked effect it will be noted that on the sphincter trace, *C*, the same respiratory movements produce only slight irregularity during the relaxation phase. The same eight respiratory movements are shown on the duodenal tracing, *A*, as definite two-phased curves. That the difference in respiratory influences upon these duodenal and sphincter records is due to a difference in tone of the viscus rather than to a difference in the thickness of wall or some other factor, is well illustrated by figure 3. Here is shown the record of an active duodenum on which the respiratory variations appear as relatively slight peaks or irregularities upon the segmental contractions. In such traces respiratory movements are of minor or negligible importance except during the relaxation phase of duodenal activity at which time they may so alter the curve as to suggest the presence of a phase of inhibition in the duodenal musculature preparatory to a following contraction. That such is not the case is shown in figure 3, and *trace 3* of figure 4, by the relation of such apparent inhibitions to the respiratory phases.

Irrespective of respiratory influences, segmental actions of the duodenum show peculiarities in the phases of the individual contractions and in the relation of these contractions to each other. This work deals specificially with those types that recurred commonly enough to be considered normal activity. The more constant types of graphic records obtained by us are as follows: *a*, Segmental contractions re-

curring at a fairly uniform rate and rising from a nearly constant tone level (figs. 3, *insert A* and 4, *trace 3*); *b*, groups of contractions, 3 to 5 in number, in which the individual contractions show variations in their height, the rate and tone level remaining nearly constant (fig. 3, *insert B*); *c*, groups of segmental contractions, 3 to 5 in number, in which

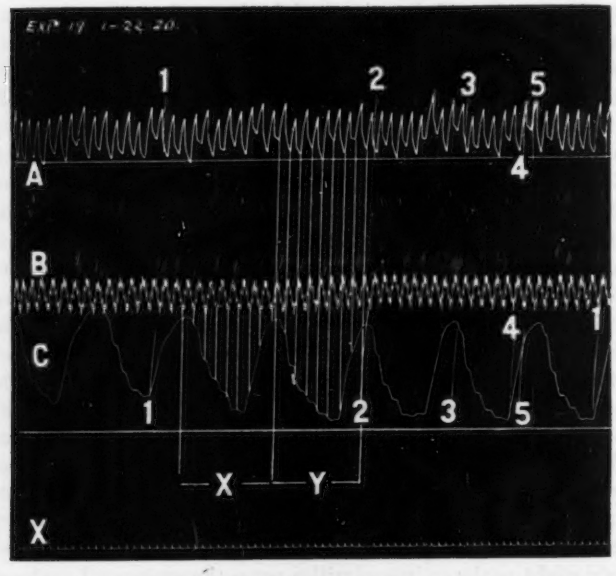

Fig. 2. Synchronous tracings of duodenal, pyloric sphincter and blood pressure activities; experiment 19; January 22, 1920. Ether used throughout the experiment. Records begun immediately following operative procedures. *A*, Trace showing the relation of changes in intra-abdominal pressure on duodenal motility; *B*, blood pressure tracing showing respiratory waves; *C*, sphincter contractions. The perpendicular lines from *X* and *Y* approximately indicate the relation of respiratory movements to blood pressure, and to actions of the duodenal and sphincteric musculature.

the contraction remainder itself varies in such a manner in the group as to give the impression of a tone wave upon which the segmental contractions are built. Such tone waves recur more or less rhythmically (fig. 4, *traces 1* and *2*); and *d*, modifications in tone level associated with variations in the appearance of segmental contractions.

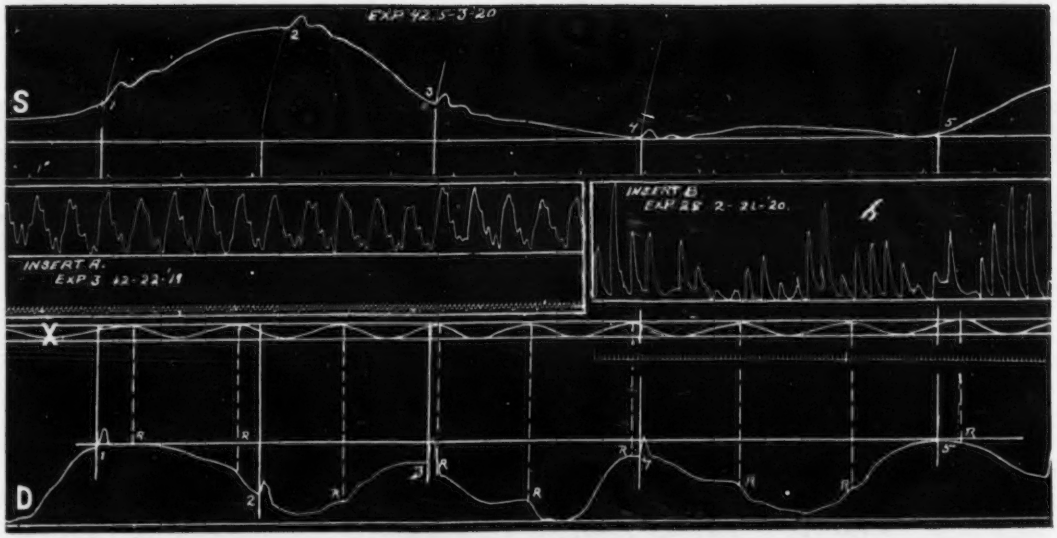

Fig. 3. Graph showing synchronous tracings of sphincter and duodenum and the influence of changes in the intra-abdominal pressure on the sphincter and duodenum. Experiment 42; May 3, 1920. Ether used throughout the experiment. *S*, Phyloric sphincter; *X*, respiration; *D*, duodenum. Time in seconds. Numerals indicate synchronous points. *R*, Relative point in the duodenal trace at the moment of greatest reaction in the intra-abdominal pressure.

Insert A: Experiment 3; December 22, 1919. Trace showing rhythmic segmental contractions of the first part of the duodenum following the administration of morphine, 10 mgm. per K. The irregularities in the waves represent the effects of respiratory movements. *Insert B:* Experiment 28; February 21, 1920. Trace of duodenal activity in a dog two weeks after the production of a gastric fistula. Tracing obtained on conscious animal.

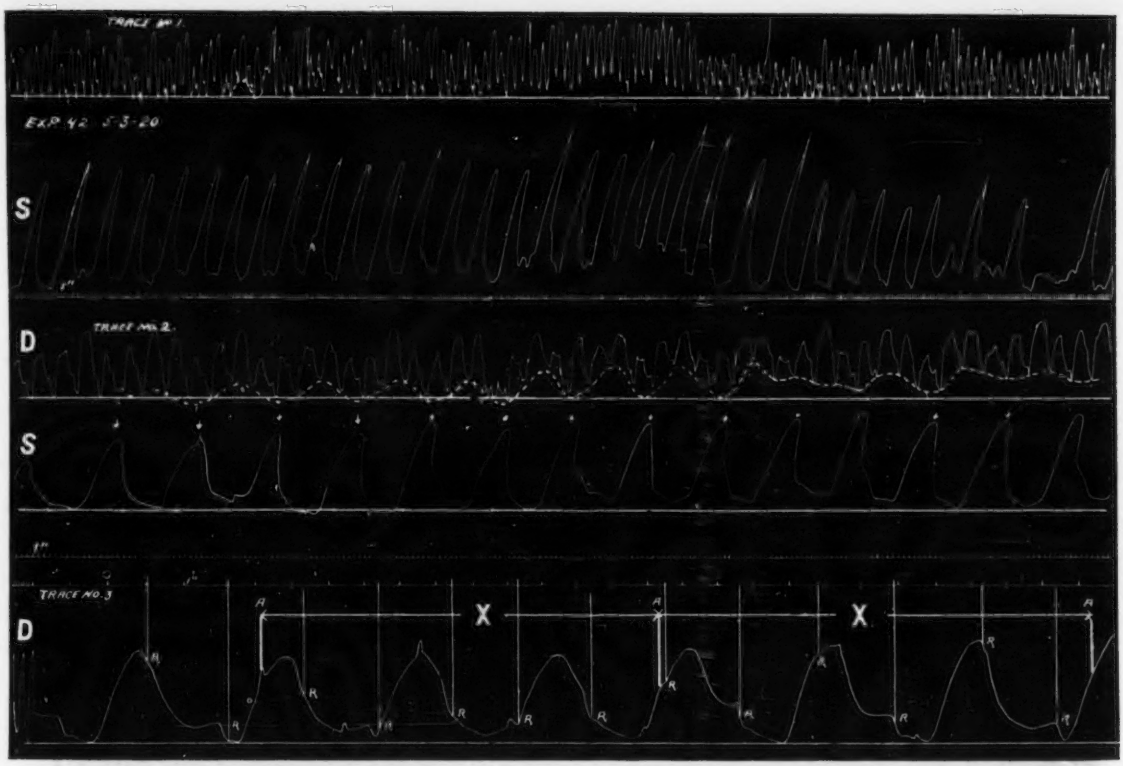

Fig. 4. Experiment 42; May 3, 1920. Ether used throughout experiment. Records begun immediately following closure of the abdomen; *D*, duodenum; *S*, sphincter. Time in seconds. Traces *1* and *2* show the relation of segmental contractions of the duodenum to the cycles of the pyloric sphincter. In *D* of trace *2*, a line is shown drawn to connect the points of greatest relaxation of segmental contractions. Note that this line forms waves the lowest points of which show a close relation to the phases of the pyloric sphincter. Trace *3* shows duodenal segmental contractions as obtained on a fast drum. *X*, Time occupied by complete sphincter cycle; *A*, crest of sphincter cycles; *R*, points corresponding to respiratory changes.

a. Rhythmic segmentations of the duodenum in which the two phases of the individual contractions are equal appear to bear no functional relationship to gastric motility. Duodenal contractions of this nature occur during an interval of constant tone level and may continue for a considerable period of time without showing essential alterations either as to rate or intensity (figs. 3 and 4, *trace 3.*). This particular type of motility was observed in the three dogs which had received morphine prior to or after operative procedures. Similar activity was observed, though less frequently, in etherized dogs and following the administration of small doses of pilocarpine. When larger doses of pilocarpine were used the duodenum usually entered upon a state of maintained contracture or tone during which segmental activity was either diminished or abolished. This latter condition was also observed preceding the act of vomiting.

b. At times segmental contractions, although arising from a nearly constant tone level, show marked alterations in the height of the individual contractions. For instance, in the *insert B* of figure 3, it will be noted that segmental contractions tend to become grouped in such a manner as to suggest periods of increased and decreased strength recurring with a certain rhythm. Such groupings, as will be shown more fully below, are more or less definitely associated with phases of motility in the pars pylorica.

c. In the third type of duodenal activity the individual segmental contractions, as stated above, are so grouped that a line connecting their points of greatest relaxation forms a definite wave. The duodenal trace of figure 4, *trace 1*, greatly resembles that of figure 2, previously described. However, in the present case the individual curves represent segmental activity and are not the result of respiratory changes; the latter show as slight irregularities of the individual contractions. The duodenal trace of figure 4, *trace 2*, shows beautifully this grouping into waves of segmental contractions. The dotted line which connects roughly the points of greatest relaxation will serve to bring out clearly the rhythmic alterations in "contraction remainder" that is under discussion. This type of duodenal activity was observed by us both in animals while under the influence of ether and those that had been permitted to recover from the influence of the anesthetic. Such tracings were often obtained over relatively long periods of time irrespective of the reception of material from the stomach.

d. Under experimental conditions duodenal tonicity was found to vary greatly, such changes being associated with alterations in rhythmic

segmentation. Immediately following the operation for the placement of an enterograph the duodenum usually showed an absence of all motility; however, motility usually reappeared within less than an hour following the completion of operative procedures. Frequently it was possible to initiate duodenal activity and states of high tonicity immediately following closure of the abdomen by moderate, but suddenly applied, distention of the duodenal balloon. A rapid and vigorous distention of the duodenum invariably resulted in the onset of retching or vomiting. Considerable fluctuation in the tone level occurred during the course of an experiment.

The tone levels at which rhythmic segmentations occur in the duodenum appear to bear a definite relationship to the activities in the pars pylorica. As may be noted in figure 5, both the extent of the individual segmental contractions and the level of their occurrence bear a close relationship to antral and sphincter actions. Furthermore, the tracings of this figure show that periods of increased duodenal motility are associated with periods of increased tonicity in the pyloric sphincter. On the other hand, periods of duodenal inactivity and loss of tone, although not associated with actual cessation of rhythmic sphincter action, do occur at times during which the tonicity of the sphincter is low. This relationship is not always present; the duodenum may show marked alterations in tonicity and the height of the individual segmental contractions independently of the sphincteric action. However, since this last type of relation between the sphincter and duodenum was seen by us only following rapid distention of the duodenal balloon, we wish simply to call attention to it. An analogous condition probably does not occur normally.

Tonicity of the duodenum appears to bear a relation to the activity in the antrum which is the reverse of its relation to the activities of the sphincter. As shown in traces *1* and *2* of figure 5, periods of elevated duodenal tonicity are associated with marked reductions in the height of the antral waves while periods of low duodenal tonicity are associated with increased antral contractions.

The type of duodenal activity that we wish to consider especially in its relation to the pars pylorica is that characteristic grouping of segmental contractions, as described in section *c*, which when taken together form definite waves (figs. 6 and 9). For the present we will refer to these as duodenal x-waves. Whether such waves represent simple tone changes or the passage of a peristaltic wave will be considered following the presentation of further data. Special attention is called

to the fact that our duodenal tracings were obtained from the first 3 or 4 cm. of the duodenum or, as previously stated, just below the region corresponding to the "cap" in man. The activity of the duodenum by which these x-waves were produced was by far the most constant type of motility demonstrated by our graphic methods. As will be shown presently, these waves bear a definite relation in their sequence to the activity of the pars pylorica.

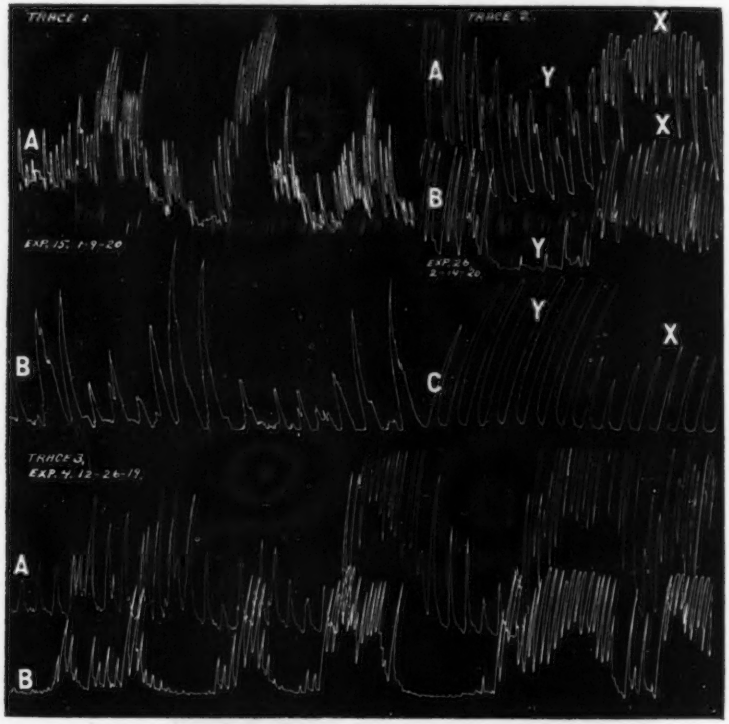

Fig. 5. Tracings showing the relation of tone changes in the duodenum to the activities of the pars pylorica. *Trace 1*, experiment 15; January 9, 1920. *A*, Duodenum; *B*, sphincter. *Trace 2*, experiment 26; February 14, 1920. No anesthesia used during registration of graph. *A*, Sphincter; *B*, duodenum; *C*, antrum. *Trace 3*, experiment 4; December 26, 1919. Conscious animal 15 hours after placement of enterograph. *A*, Sphincter; *B*, duodenum.

Note that periods of increased duodenal tone are associated with depressed antral and augmented sphincter activity.

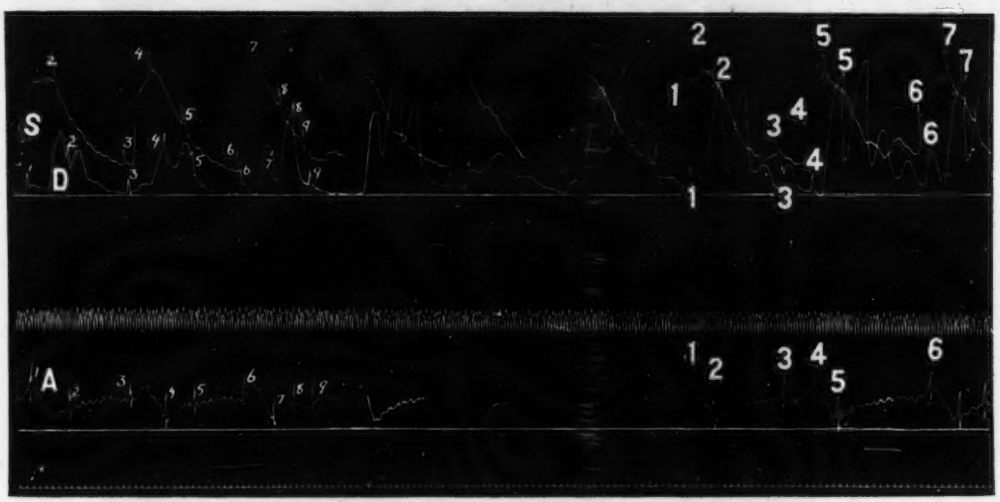

Fig. 6. Tracing showing simultaneous activities in the antrum, sphincter and duodenum. Numerals indicate synchronous points. Experiment 22; January 30, 1920. Ether used throughout experiment. Triple, spindle-enterograph. *S*, Sphincter; *D*, duodenum; *A*, antrum.

Note the relation of duodenal activities to those of the pars pylorica.

Relation of antral to duodenal activities. Joseph and Meltzer were the first to call attention to the fact that activity in the descending part of the duodenum in rabbits is associated with inhibition in the gastrium. They state in their brief report: "During each contraction of the pyloric part of the stomach the duodenum stops its rhythmic activity and loses its tone, only to resume both again as soon as the contraction of the stomach passes off" (3). The details of these experiments were not given and up to the present time have not appeared in

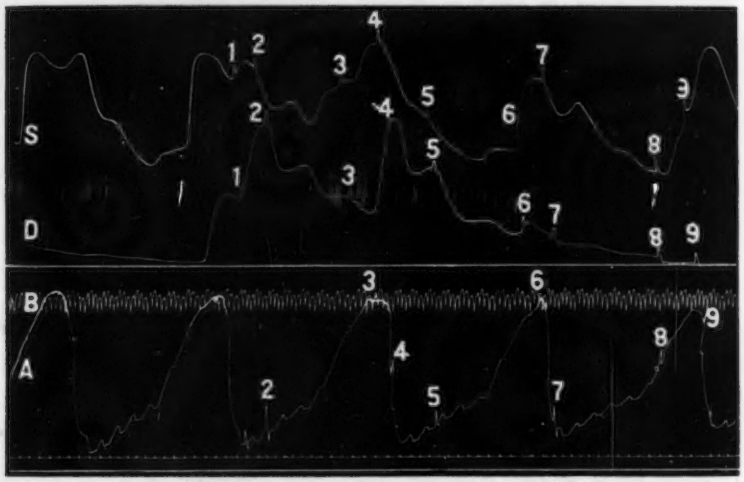

Fig. 7. Tracing showing the relation of tone changes and segmental contractions to the activities of the pars pylorica. *S*, Sphincter; *D*, duodenum; *B*, blood pressure; *A*, antrum. Time in seconds. Experiment 22; January 30, 1920. Ether used throughout experiment. Spindle, triple-balloon; antral chamber large. Numerals indicate synchronous points.

the literature. However, Joseph informs us that their results were obtained by means of small balloons anchored in the antrum and descending duodenum respectively and that observations were made at intervals up to 30 days after the operations. No mention is made in their report of a possible relation between sphincter and duodenal activities.

The present investigation of the relation of duodenal to antral activity is one of the sequellae of our studies on the motility of the pyloric sphincter (1), (2). This work was begun and practically completed

before our attention was directed to the above mentioned contribution. We take this opportunity to thank Joseph for his interest in the present investigation.

Simultaneous tracings obtained by the use of a double-chambered enterograph, one chamber of which occupies the antrum, the other the first part of the duodenum, show that the duodenal x-waves are intimately associated with the activity in the antrum. The specific re-

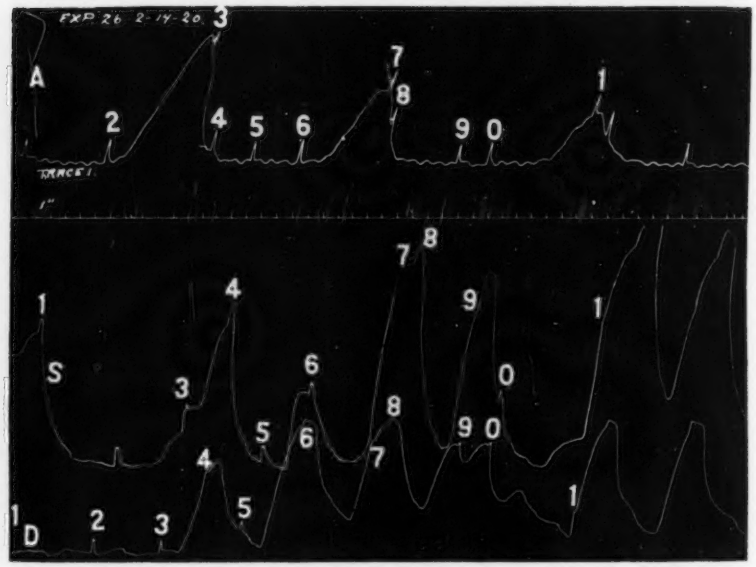

Fig. 8. Tracing showing the functional relation of the duodenum to the pars pylorica. Experiment 26; February 14, 1920. Records obtained on conscious animal. *A*, Antrum; *S*, sphincter; *D*, duodenum. Time in seconds. Numerals indicate synchronous points.

Note that the sphincter responds to each action of the duodenum.

lationship of these two portions of the gut is shown in figure 6 and diagrammatically in figure 9 which represents the average of a series (27) of tracings. For the sake of clearness and brevity, this relation between duodenal and antral activities may be considered from figure 9. In this drawing the period for a complete antral cycle, curve *A*, is shown as 18 seconds. Rhythmic segmental activity of the duodenum is shown by curve *DS*. Here the 5 complete segmental contractions,

A, *B*, *C*, *D* and *E*, each of which occupies approximately 3½ seconds, although the height of the individual contractions varies considerably, group themselves in such a manner as to suggest a wave. A line connecting the points of greatest relaxation (curve *DP*) in the series of segmental contractions describes an arc which, beginning at *X*, reaches its greatest height between points *2* and *3*. From this point the curve gradually descends to reach the level of its origin (point *1*), which constitutes the level from which the individual contractions are initiated during the formation of the x-wave. The duodenal x-wave demonstrates

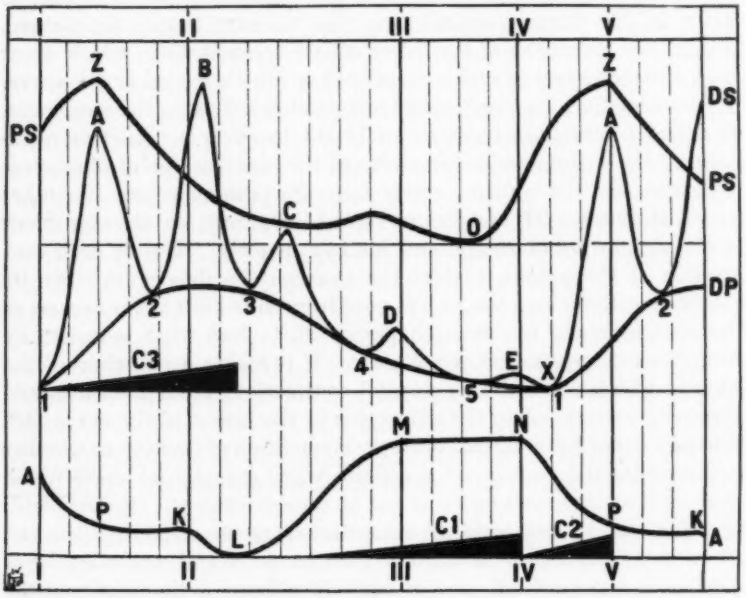

Fig. 9. Diagram showing the average relation of the sphincter to the antrum and duodenum. *PS*, Pyloric sphincter curve; *DP*, duodenal peristaltic curve; *DS*, duodenal segmental contractions; *A*, antral curve. Time indicated in seconds. *C 1*, *C 2*, *C 3*, diagrammatic representation of the movement of chyme during its passage from the antrum into the duodenum.

Note that the first segmental contraction of the duodenum, *A*, reaches its highest point synchronous with the sphincter and at a time of relaxation of the antrum. Also that the duodenum demonstrates its greatest degree of relaxation, *X*, after the antrum has ceased contracting and during the period of contraction of the sphincter. The maximal height of the peristaltic wave in the duodenum occurs while the antrum is relaxing prior to the wave of contraction, *L*.

least activity at this point; approximately one second after commencement of relaxation in the antrum (point *N*). The ascent of the first segmental contraction following maximal duodenal relaxation is rapid and reaches its height, *A*, at a time of nearly completed relaxation of the antrum (point *P*, curve *A*). The phase of relaxation of contraction *A* is interrupted at *2* by the appearance of contraction *B*. The three remaining segmental contractions show a progressive diminution of intensity. In contractions *A* and *B* the relaxation phases are not equal to the contraction phases while in curves *C*, *D* and *E* the relaxation phase is the more pronounced. The duodenal activities, as shown in this diagram, constituting what we have termed the x-wave, is therefore composed of graded rhythmic segmentations which occur from different levels in such a manner as to form a second or *DP* curve. This latter curve or wave, forming immediately following the completion of antral contraction, increases in height to cover a period of relaxation of the antrum and returns toward the base line—point of greatest relaxation—as the antrum enters upon its positive phase. In other words, the curves *DP* and *DS*, presented in figure 9, are the equivalent of the statements of Joseph and Meltzer; namely, "During each contraction of the pyloric part of the stomach the duodenum stops its rhythmic activity and loses tone, only to resume both again as soon as the contraction of the stomach passes off." Just what is meant by "tone" in this quotation is not clear. If it means a reduction of the level at which diminished segmental contractions occur then it would appear to correspond to the differences in the height of the curve *DP*. Whether curve *DP* constitutes simply a condition of tonicity or whether it represents the passage of a definite wave (peristaltic) upon which segmental actions are grafted is not at present evident. It is evident, however, that the height of the segmental contractions do show marked alterations which bear a definite relation to antral activities. For instance in the duodenal trace of *insert B* of figure 3, the heights of the individual segmental contractions tend to form graded series although the apparent tonicity of the duodenum remains constant. On the other hand, such alterations of height in the individual segmental contractions may be associated with incomplete phases of relaxation or form weak x-waves. In either case the higher segmental contractions appear following the beginning of relaxation in the antrum (fig. 6). From this alone it might be argued that the duodenal x-wave represents nothing more than a tone wave, that is, that increased tonicity permits of segmental contractions of greater strength while reduced tonicity

(within limits) results in a depression of segmental contractions. However, the tracings of figures 7 and 8 would seem to show that the duodenal x-waves are not tone waves. For instance, in figure 7 in which two x-waves appear, the second wave originates at a height (tone level) which is much greater than that of the first although the two waves bear the same relation to the activities of the antrum as shown by the synchronous points marked by numerals. In figure 8 rhythmic segmental contractions beginning in an inactive duodenum, show such changes in the level of their origin as to suggest an increased duodenal tonicity over a period of two complete antral cycles. However, it should be emphasized that segmental contractions in the duodenum may occur independently of either tone changes or x-waves, and even in the absence of both. It appears, therefore, that tonicity of the duodenum may vary considerably without altering the relation of the duodenal x-waves to motility of the antrum, and that simple segmental contractions, which are not associated with alterations in the antrum, can occur from a constant level.

Relation of sphincteric to duodenal activity. Sphincter contractions, as shown in a previous paper, reach their highest point at a time when the antrum is rapidly relaxing or is completely relaxed (1). The first segmental contraction which occurs in an x-wave reaches its height simultaneously with that of the sphincter (fig. 9, *A* curve *DS*, and *Z* of curve *PS*); i.e., the duodenum and sphincter execute contractions which are maximal at the same moment although the duration of contraction of the sphincter is greater than that of the duodenum. The second individual contraction, *B*, usually reaches a greater intensity than the first but this and the subsequent contractions occur during the period of relaxation of the sphincter. The first segmental contraction, as previously shown, is initiated approximately one second after the beginning of relaxation of the antrum and from the point of greatest duodenal relaxation. On the other hand, the sphincter begins its contraction approximately $2\frac{1}{2}$ seconds prior to the first segmental contraction, *A*, or while the antrum is still at the height of its contraction phase. Hence the sphincter has reached a high degree of contraction before the antrum begins its relaxation phase and before the duodenum begins a new x-wave or at the time of completion of a previous wave. Therefore, the period of greatest duodenal relaxation from which the first segmental contraction, *A*, occurs coincided with the period of contraction of the sphincter. The second and third, *B* and *C*, segmental contractions occur from higher levels than the primary or first segmental

contraction; the remaining two contractions, *D* and *E*, are greatly reduced in height and occur from successively lower levels. Therefore, curves *DP* and *DS* (x-wave) reach the greatest degree of contraction between 3 and 4 seconds following completion of the sphincter's positive phase, or during the first portion of its period of relaxation. From this point the heights of the curves *DP* and *DS* gradually diminish to finally reach the point of greatest duodenal relaxation and inactivity, point *1*. During this period of decline in the x-wave, especially curve *DP*, the sphincter is rapidly relaxing or quiescent while the antrum is entering upon its positive phase; i.e., a positive phase in the antrum begins during the relaxation phase in the duodenum and sphincter. In other words, the duodenum demonstrates an inactive phase during the positive phase of the antrum and sphincter which reaches its greatest degree of relaxation (inhibition) immediately following completion of the positive phase of the antrum and when the sphincter has begun its positive phase (figs. 4, 6, 7 and 9).

The results of graphic methods, as described above, show that a fairly definite relation exists between the rhythmic grouping of segmental contractions in the duodenum and the phases of motility in the pars pylorica. An interpretation of these x-waves and of their functional relation to the stomach must, at present, be guarded for the reason that the impression now prevalent concerning the independence of gastric and duodenal activity offers no support to our findings (4). As already pointed out, it is difficult to explain these waves in the duodenum upon the assumption of tone changes. Moreover, if they are tone changes it becomes necessary to explain the origin of such tone changes. *A priori*, there is no reason to assume that the duodenum undergoes tone changes which have the same rate as peristaltic waves in the stomach, and at the same time bear no intimate relation to such waves, especially when material is not permitted to reach the duodenum from the stomach. The character and constant time relation of the x-waves to the acts of the pars pylorica is such as to suggest a continuance of the contraction process which passes over the antrum and sphincter. If these x-waves are to be considered modified segmental contractions then they must be differentiated from the usual segmental contractions of the duodenum because they require a greater period of time for their completion than is commonly thought to be covered normally by rhythmic segmental contractions. Moreover, during the passage of the contraction process over the first portion of the duodenum, rhythmic segmentation continues although the phases of

the individual contractions are markedly altered. At times rhythmic segmentations occur unassociated with alterations in height and without being grouped into rhythmically repeated cycles. Consequently two fundamentally different factors are operative in the production of the x-waves. If rhythmic segmentations can occur unassociated with alterations in intensity and tone level, and if these same rhythmic segmentations can be so altered as to form rhythmically appearing groups of contractions because of activities of the stomach, then it must either be assumed that the appearance of the factor which causes this grouping of segmental contractions excites all types of motor responses, thereby causing alterations in segmental phenomena, or that increased segmental activity gives rise to the formation of x-waves. This latter assumption appears untenable for without the appearance of the factor which causes the production of the x-waves the segmental contractions remain of uniform height and occur from a constant level. Therefore we may assume, at least tentatively, that the factor which causes the grouping of segmental contractions in the first part of the duodenum is a peristaltic wave which has passed into it from the stomach or that the duodenum has been excited because of gastric motility.

As suggested above, rhythmic segmentation may occur independent of, or intimately associated with, gastric contractions. It has been shown that periods of greatly increased tonicity associated with heightened rhythmic segmentations are also associated with a depression in the height of antral contractions and a marked increase in the tone level at which the sphincter demonstrates its rhythmic contractions. Not infrequently the pyloric sphincter responds more or less to each duodenal motor activity. Hence not only may the activities of the duodenum be influenced by the stomach, but also may the activities of the stomach be influenced by those of the duodenum. If, for example, in an exposed preparation, the finger is placed within the lumen of the pyloric sphincter, one notes that with the initiation of a positive motor phase in the duodenum—outer portion of the "cap" region—there is a decided compression on the finger because of the sphincter's contraction. Direct observation at such times shows that the sphincteric contraction occurs immediately following the first dimpling on the greater curvature of the duodenum. Similar results may be obtained graphically by placing a pylorograph in the lumen of the sphincter and noting, at a time of inactivity (complete relaxation), the effect of strong stimulation of the duodenum along its greater curvature. In such an experiment stimulation of the duodenum invariably excites the sphincter to demonstrate motor phenomenon, the period of contraction of which

is short, the phase of relaxation more or less maintained. Three such responses of the sphincter to duodenal stimulation are shown in figure 6, page 136, of a previous paper (1), all of which were obtained from a quiet stomach and duodenum immediately following operative procedures for the placement of a pylorograph. Similar motor relations between the sphincter and duodenum may be seen during the synchronous registration of motility in the antrum, sphincter and duodenum. Such a condition is well shown in figure 8. Here the contractions of the sphincter are of varying height and duration. Prior to the appearance of duodenal activity the sphincter demonstrates only movements which bear the usual relation to the antrum. The same relation is continued throughout the trace as indicated by points *4*, *8*, *1*, however, following the appearance of duodenal activity the sphincter is seen to respond to each duodenal contraction occuring during the inactive phase of the antrum, points *6* and *9*, i.e., the sphincter responds not only to the reduced antral contractions but also to the individual duodenal contractions. Here two complete antral cycles are associated with four complete sphincter and duodenal cycles, that is, the appearance of duodenal activity is such as to cause an extra sphincter response during an antral cycle (curves *6* and *9*). These extra sphincteric contractions are usually less pronounced than those bearing the usual relation to antral activity although the maximal height of contraction of the duodenum occurs synchronously with the point of maximal contraction of the sphincter in each case. However, this relation between sphincter and duodenum seems to bear some relation to the degree of activity in the antrum; usually antral contractions are reduced in height although they occur rhythmically.

In figure 7 the duodenum demonstrates two complete waves of action, each wave being associated with three segmental contractions, each of which in turn is associated to some degree with alterations in the sphincter, although such alterations occur as minor modifications of the response to the antrum. In this trace a second wave of the duodenum and the corresponding sphincter wave occur from a higher level than the one just preceding or the two immediately following. This condition is analogous to the more marked duodenal-sphincter relation shown in figure 5. With the development of a constant repetition of peristalsis in the duodenum and an active antrum the irregularities in the sphincter curve become greatly reduced or abolished (fig. 6). It therefore appears that a relatively quiet antrum may occur during active duodenal motility, also that periods of increased duodenal activity may be associated with increased motor activity of the sphincter.

DISCUSSION

Mering, 1893, (5) advanced the theory that the filling of the small intestine reflexly retarded the emptying of the stomach. This view was shared by Schicker in 1911 (6). Marbaix, 1898, (7) contended that it was the duodenum which exerted the influence for the replete intestine on the closing of the pylorus. According to Cannon (9), the degree of gastric acidity determines the reflex opening of the pylorus. Cole (10), from his radiographic studies of the human stomach and duodenum, concluded that the duodenal-sphincter reflex from the replete intestine affects not the pyloric sphincter as believed by Marbaix (7) but the the contractions which withdraw the chyme from the "reservoir cap." This process Cole considers is similar to the one by which the duodenum is replenished from the stomach.

The observations and conclusions of Cole seem to us worthy of note, both because of their clinical and theoretical aspects. It seems logical to assume that the degree of receptivity of the small intestine is as great a factor in the emptying of the "cap" as the motility of the stomach is in filling this region. By way of illustration we may cite the observation that one dog starved 18 hours emptied its stomach of an 8-ounce barium mixture in 80 minutes, while a second meal of the same amount was not completely removed at the end of 2½ hours. Analogous results were observed in human subjects.

The results of our present and previous work show that the acts of opening and closing of the pyloric sphincter are to a great extent dependent upon the motility of the structures lying on either side. That is, the sphincter acts as a functional link in the chain of forward conduction of material from the stomach, thereby aiding both gastric and "cap" evacuation. Our graphic results are in agreement with the radiographic findings of Cole regarding the relation of the sphincter to the duodenum. That is, the "reservoir cap" receives the chyme during the active phase of the antrum, material being held in this region at the time of relaxation of the antrum because of contraction of the sphincter. The duodenum, just beyond the "cap, demonstrates its greatest degree of relaxation (loss of tone) at the close of the positive phase of the antrum and at a time of rapid contraction of the sphincter. However, following this period of maximal depression, the duodenum develops a positive phase as evidenced by the appearance of an x-wave the first segmental contraction of which reaches its maximum along with that of the sphincter or at a time of marked relaxation in the antrum. Thus the positive phase of the duodenum is well initiated at

the moment of beginning sphincter relaxation. Therefore, this positive phase in the duodenum associated with a powerful initial segmental contraction, both of which are initiated during the period of sphincter contraction, act in such a manner as to supplement the activity (positive phase) of the sphincter.

For the sake of clearness let us suppose that a single bolus of food is presented to the region of the sphincter during the positive phase of the antrum, also that the antrum, sphincter and duodenum constitute a tube similar to any portion of the intestine demonstrating peristalsis; then, according to "the law of the intestine" there should be a state of relaxation in the pre-antral region (behind the constricting band), inhibition of the sphincter and lower segments and a positive band of constriction behind the bolus or in the antrum proper. Inasmuch as peristalsis is a progressive phenomenon, the bolus would be expected to pass the sphincter and enter the portion below, namely, the duodenum, the sphincter closing as the result of the positive phase of the peristaltic wave reaching this structure. The antrum and sphincter would now demonstrate their inactive phases while the positive wave would pass over the first part of the duodenum.

Such a hypothetical case as the one just given finds experimental substantiation in our results. The main difference between the hypothetical case and the results of experimental methods is that in addition to the passage of a peristaltic wave over the duodenum there is associated with it the phenomenon of rhythmic segmentation. A further discrepancy appears in that the sphincter not only responds as a functional unit in the progressive movement of a peristaltic wave but also, at times, to each rhythmic contraction in the duodenum. This, however, does not disprove the above statement for, as will be recalled, a true peristaltic wave is one which involves contraction above the food mass and relaxation—inhibition—below.

If the duodenal musculature, because of excitation or because of distention, responds as any other portion of the gut, i.e., inhibition at point of excitation and contraction cranialward, then a band of constriction should appear in the portion next higher, that is, in the sphincter, because this is the first definitely contractile portion behind the bolus in the "cap" region. That the sphincter may respond to individual, rhythmic, duodenal contractions has already been shown. That the sequence of actions in the duodenum as related to gastric motility is not the result of distention of the duodenum because of material delivered to it is evidenced by the fact that this same relation holds

for the motility of the empty stomach and duodenum and also for preparations in which material is prevented from leaving the stomach because of the presence of an enterograph.

If, as contended by Cole, duodenal receptivity controls the evacuation of the "cap" through duodenal peristalsis rather than the evacuation of the stomach through a pyloric sphincter, then it would appear as though the sole function of the sphincter and antrum, as far as motility is concerned, is to assure the filling of the cap. However, the statements of Cole are reconcilable with the present work. Granting that the "cap" is evacuated by duodena peristalsis—a statement we agree with—such peristaltic movements would of necessity force material back into the antrum save for the checking influence of a contracting sphincter. As shown above, this duodenal wave of peristalsis which withdraws material from the "cap" arises, as far as the duodenum is primarily concerned, in the outer portion of the "cap" along with a powerful segmental contraction at a time when the sphincter is rapidly mounting to the height of its positive phase. Once the peristaltic wave (x-wave) is formed in the duodenum there is no apparent reason for assuming that the sphincter need continue its positive phase. Hence it may be assumed that the sphincter aids not only in the processes which fill the "cap" because of gastric evacuation, but also in the processes which fill the duodenum from the material in the "cap."

Alvarez (4) states: "It is not generally known that there is a fairly complete connective-tissue barrier between the muscle of the antrum and duodenum. Ordinarily there are only a few bundles from the longitudinal coat which pass over. This explains the fact that gastric waves do not run over onto the duodenum. Graphic records show that some influences may pass over to start peristaltic rushes in the bowel, but the deep waves, visible to the unaided eye, certainly stop at the barrier" (p. 614). Again: "There is considerable evidence for the view that the rhythmicity of the primitive gastro-intestinal tube is graded downward from the pharynx to anus much the same as the rhythmicity of the primitive heart tube is graded from the venous to aortic end," however, "in mammals specialization in the functions of the stomach has been accompanied by specialization in the muscle and a loss in the rhythmicity." Possibly the differences between the slow rate of the antrum and the more rapid rate in the duodenum and at the incisuria angularis of the stomach are to be explained on the basis of this muscular modification.

The "connective-tissue barrier" between the sphincter and duodenum is not as extensive in the dog as the above statement leads one to believe. In this animal it is true there is considerable connective-tissue among the bundles of muscle fibers constituting the terminal antrum and sphincter; however, numerous muscle fibers are found connecting the antrum with the duodenum. That connective-tissue should be found in abundance in this region is not surprising in view of the fact that connective-tissue usually occurs in relatively large amounts where it is necessary to supply large blood vessels. The statement that "gastric waves do not run over onto the duodenum" save "to start peristaltic rushes in the bowel" appears to contradict itself. However, the latter statement would seem more logical than the first in the light of the author's own hypothesis. The apparent stoppage of the gastric wave at the sphincter may be assumed, as suggested above, to result because of the degrees of specialization of the musculature beyond this point. In the light of our work it must be assumed that the degree of specialization of the musculature in the sphincter and duodenum of dogs is not sufficient to cause cessation of a progressive movement over the stomach at the pyloric sphincter.

Our graphic results indicate that the factors exciting waves of peristalsis in the stomach are more or less directly responsible for peristalsis in the duodenum, the phases of which bear a definite relation to those of the stomach. Inasmuch as our graphic records show a progressive series of events in the antrum, sphincter and duodenum which bear a constant and definite time relation to each other, we believe that the activities of these three parts conform to the principle of the "law of the intestine." Therefore our results, if correctly interpreted, appear to lend considerable weight to the "gradient theory of the gastro-intestinal tube" as evolved by Alvarez inasmuch as they show a progressive series of events in the musculature of the stomach and the first part of the duodenum.

SUMMARY AND CONCLUSION

The outstanding features in the action of the duodenum in relation to those of the pars pylorica may be briefly summarized as follows:

1. The pyloric sphincter, like the antrum proper, demonstrates cycles of rhythmic motility. These cycles are of the same duration as those of the antrum; however, their phases are so placed that they functionally supplement the activities of the antrum. A phase of

inhibition (maximal relaxation) manifests itself during the height of antral contraction. Following this, and while the antrum is finishing its contraction, the sphincter begins its positive phase and reaches the height of its contraction when the antrum is rapidly relaxing or is relaxed. Following this the sphincter relaxes to ultimately reach its point of greatest depression preparatory to a second positive phase.

2. The rhythmic activities of the duodenum, as first shown by Joseph and Meltzer, like those of the sphincter, bear a definite and constant relation to the various phases of the antrum. Peristaltic waves in the duodenum are associated with groupings or segmental contractions the first of which usually has its origin at the same time the positive phase of a peristaltic wave reaches the duodenum, or better, perhaps the appearance of a contraction wave in the duodenum excites rhythmic segmental contractions in the region in which they were previously depressed or abolished because of a preceding wave of inhibition. The peristaltic wave begins in the duodenum approximately one second after the antrum has begun to relax and at a time of rapid contraction of the sphincter and reached its height during the phase of rapid relaxation of the sphincter and while the antrum is entering upon its phase of greatest depression.

3. The observations recorded above shown that the antrum, pyloric sphincter and first part of the duodenum in the dog each possesses definite rhythmic cycles which bear a constant relationship and show a dependence upon each other, that is, parts excited cranialward travel caudalward to excite lower segments. The three portions, therefore, show progressive motility which occurs in a definite sequence and in a manner which we may believe normally causes material to leave the stomach and to be delivered into portions of the gut below the first portion of the duodenum.

4. The sphincter may be excited to cover a phase of motility in the duodenum occurring during the inactive phase of the antrum. The sphincter, therefore, may act not only in relation to the antrum to aid in the propulsion forward of material from the stomach, but also as a barrier to the regression of chyme during the presence of a positive phase of the duodenum.

5. In conclusion, it may be assumed that the progressive nature of the cycles of events in the three portions of the gastro-intestinal canal studied demonstrate "the law of the intestine" i.e., a progressive band of constriction preceded by inhibition and followed by relaxation.

BIBLIOGRAPHY

(1) WHEELON AND THOMAS: Journ. Lab. Clin. Med., 1920, vi, 124.
(2) WHEELON AND THOMAS: This Journal, 1921, liv, 460.
(3) JOSEPH AND MELTZER: This Journal, 1910, xxvii, p. xxxi of Proc. Amer. Physiol. Soc.
(4) ALVAREZ: Amer. Journ. Med. Sci., 1919, clviii, 609.
(5) VON MERING: Verhandl. d. Cong. f. innere Med., 1893, xii, 471.
(6) SCHICKER: Deutsch. Arch. f. klin. Med., 1911, civ, 566.
(7) MARBAIX: Cellule, 1898, xiv, 249.
(8) CANNON: This Journal, 1898, i, 359.
(9) CANNON: This Journal, 1907, xx, 283.
(10) COLE: This Journal, 1916, xlii, 618; Journ. Amer. Med. Assoc., 1913, lxi, 762.

THE ACTIVITIES OF THE INTESTINAL MUCOSAL MOTOR MECHANISM

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Much has been written about the motor activities of the intestinal tract. A survey of the literature shows that with a few exceptions these writings have been based upon experiments and observations involving only the outer, the circular and longitudinal muscles of the intestine. Little attention has been given to the activities of the muscularis mucosae. Inasmuch as in all higher animals this musculature is well developed, it would seem necessary to assume that it manifests some sort of activity and contributes an essential rôle to the normal physiology of the small intestine.

At this point it is well to recall that some of the fibers of the muscularis mucosae extend into the villi, consequently mucosal motor activities in the intestine would imply movement of the mucosa as a whole, or in limited areas, and also movement of the individual villi.

A number of earlier workers have reported observations on movements of the intestinal mucosa. Lacauchie (1) observed a shortening of the villi after death. Gruby and Delafond (2) observed shortening, lengthening and lateral movement in the living animal. Brueke (3) was among the earlier students of the musculature of the villi and ascribed changes in shape and position in them to muscular contraction and relaxation. Exner (4) studied the reactions of the intestinal mucosa to mechanical stimulation and found it to respond sharply to such a procedure. Gunn and Underhill (5) recorded and studied the movements of the mucosa of the cat's small intestine, using strips from which the outer musculature had been removed. They were able to record a definite rhythmical movement. Hambleton (6) observed, by using a binocular microscope and watching lighted fields of intestinal mucosa in intact animals, shortening, lengthening and lateral movement of the villi.

The present study was undertaken for the purpose of inquiring more fully into the types and varieties of movement manifested by the mucosa

of the dog's small intestine, and to investigate the nature of and the control of the mechanism involved.

METHODS AND MATERIAL. It is practically impossible to record movements of the intestinal mucosa, especially the individual villi in the intact animal, by mechanical means. One might think of photographic methods, a series of pictures showing the villi in different positions and shapes. Owing to the magnification required as well as the intensity of light, this method, at least in our experience, has proven next to valueless.

We have adapted the method of observing the field through an extension binocular microscope, using a magnification of 25 diameters, the field of observation lighted by a small arc light. The results are recorded as dictated by the observer, or in some instances the villi rhythm is recorded by means of a key and signal magnet.

With few exceptions dogs have been used. Cannon (7) has shown that the motor mechanism of the outer musculature of the intestine is relatively long-lived. Not so with the mucosal mechanism. It is extremely sensitive to circulatory changes, anemia in particular, to thermal changes, and to mechanical and chemical irritation. Our experience has been that the mechanism holds up better in dogs than in cats, rabbits and goats.

Ether depresses the irritability of the mucosal motor mechanism. For this reason we have partially decerebrated most of our animals. After preliminary ether anesthesia the parietal bone is rapidly drilled through, making the hole large enough to admit a Mall probe. The anterior half of the cerebrum is thus destroyed. The animal remains quiet without further ether, maintaining a normal respiration and blood pressure. Occasionally, if the brain injury has been extensive, respiration fails, accompanied by a fall in blood pressure. Although these latter animals may be kept alive for several hours by artificial respiration, it has been our experience that the mucosal motor mechanism seldom shows anything like normal activity.

After decerebration the abdomen is opened by a median longitudinal incision and a loop of the small intestine pulled up. An incision about 5 cm. long is made through the wall opposite the mesenteric attachment. The loop is then slipped through a slit in a small board and the edges of the gut are pinned out so as to present a flat mucosal field for observation. The board is held in position by a burette clamp attached to stand. The field is lighted by means of an arc lamp, the binocular adjusted, and the preparation is ready for observation.

PROCEDURE AND EXPERIMENTS. *Mechanical stimulation.* When the mucosal surface of the small intestine was touched or stroked with a probe or fine glass rod there appeared a depression or groove at the point of contact followed quickly by an anemia of greater or less intensity, depending apparently, within certain limits, upon the intensity of the stimulus. In case there was no further mechanical disturbance a normal appearance was resumed in from 30 seconds to 1 minute. With the field lighted and with magnification, in addition to seeing the almost disappearance of the blood from the villi capillaries, it was clear that two factors were involved in the formation of the pit or groove, the shortening of the individual villi, and a contraction seated apparently in the deeper portions of the muscularis mucosae.

The response to mechanical stimulation was sharpest in new preparations, then gradually diminished to a degree of sluggishness making the preparation useless for experimental purposes, or disappearing entirely. Thirty minutes represented about the average life of the mechanism under our working conditions. Drying and lowering the surface temperature were apparently factors involved, for the life of the preparation could be considerably prolonged by frequent irrigation with isotonic saline or dextrose at body temperature of the animal. That this change of condition was a local one is evidenced by the fact that, with the animal in good condition, a new preparation just a few inches from the old one manifested normal reactivity.

The irritability and activity of the intestinal mucosa were the greatest in the duodenum and upper third of the jejunum. The duodenum and jejunum usually manifested considerable spontaneous activity when the fields were first prepared, or in case the mucosa was quiet the response to various types of stimuli was very sharp. In the ileum one occasionally saw a few scattered villi in activity, more commonly not. Vigorous stimulation in the ileum brought on a fair grooving and ridging reaction but scarcely any reaction on the part of the individual villi.

A study of the progressive decrease in irritability to mechanical stimulation revealed the fact that the tops of the villi were the first points to diminish in irritability. When the gut was first opened and the top of a villus was touched with a fine glass rod it quickly retracted, became anemic and then lengthened. In many instances there was restlessness for a few seconds, a swaying movement, then it became quiet, or resumed its regular rhythm of what we might designate as spontaneous movement. If care was taken not to disturb the villus violently with the glass rod the reaction as a whole did not involve the neighboring

villi, but if the tip of the rod was applied in the neighborhood of the base of the villus or pushed deep into the mucosa, a characteristic anemic pit or groove appeared. As the preparation grew older the top of the villus gradually lost its sensitivity, the villus reacted more slowly and less completely, then manifested only a swaying movement, and finally gave no reaction at all. The deep mucosal reaction usually persisted after the individual villi ceased to respond.

The results of electrical stimulation were similar to those obtained by mechanical stimulation, the type, extent and duration of the reaction depending upon the strength of the stimulus applied.

Thermal changes. It is a general rule that the irritability of living tissue increases up to a certain limit with a rise in temperature, and diminishes with a fall. Our attention was first called to the marked sensitivity of the intestinal mucosal motor mechanism in an accidental way. During the course of an observation of a field manifesting active villi movement, one of the assistants opened the door leading into the laboratory. This created a strong draft of cold air. In an instant all movement ceased. The door was closed and movement was gradually resumed. On checking this point it was found that the same effect could be produced by blowing the breath over the field, or by fanning. The same fact was demonstrated by irrigation with isotonic saline or dextrose at different temperatures. In many instances a sluggish field was revived by irrigation at body temperature or slightly above. Likewise active fields were made sluggish or movement actually stopped by irrigation below body temperature. In determining this point care was taken to avoid variations in mechanical stimulation due to the flow of the fluid over the field.

Local applications of irritants and drugs. These experiments were carried out primarily to determine the reactivity of the mucosal motor mechanism to chemical stimulation. Cantharides, thymol, menthol, alcohol, peppermint oil, croton oil, colocynth and magnesium sulphate were used. One per cent cantharides in saline was used. The amount of cantharidin in such a preparation is small and its solubility slight. One drop placed on the tongue produced a slight tingling sensation. The tincture was avoided in order to eliminate the complicating effect of the alcohol. Both menthol and thymol are slightly soluble in water. Saturated solutions at 39°C. were used, and also small crystals were laid on the surface. Peppermint oil was used in the form of a saturated solution in saline. Croton oil was used in the form of an emulsion 10 drops in 100 cc. of saline to which had been added a few drops of a

solution of egg albumin. One per cent compound extract of colocynth in saline was used. In this preparation several substances which are irritating to the mucosa are present and the effect in all probability is a complicated one. Magnesium sulphate was used in different strengths, 2.5 per cent, 3.5 per cent, and also crystals were placed on the surface. Alcohol was used in strengths of 1 and 10 per cent.

All of the above named preparations augmented the activity of the villi, although not to the same degree. Peppermint, menthol, magnesium sulphate and the colocynth preparatious were the most active. Cantharides produced a restlessness, swaying movement, but did not to any marked extent increase rhythmical movement. Croton oil was the least effective. This might be expected, inasmuch as no provision was made for the splitting of the oil, the action noted being due to the small amount of free acid originally present. One per cent alcohol (ethyl) acted as a mild irritant; 10 per cent alcohol was injurious and led to a rapid loss of irritability of the villi.

There is no necessity for detailed protocols of these experiments. There was no essential difference in the types of reaction produced. The variations in results seemed to be due to the variations in the concentrations of the active substances. With mild irritation the villi, if quiet at the beginning, manifested only restlessness; if active, a slight increased activity. More intense irritation led to vigorous activity on the part of the villi. In some instances this might be designated as stormy. In these cases the villi movement was more or less associated with a deeper mucosal movement. Also with mild irritation one seldom noted any change in the outer musculature, but with vigorous stimulation rhythmical movement was set up, however coming on later than the increase in mucosal activity.

In this connection should be mentioned a series of experiments on irrigation of the musosa with hypotonic and hypertonic solutions of sodium chloride and dextrose, and also irrigation with distilled water. Hypertonic solutions of salt and dextrose produced a clear-cut shrinking of the villi, hypotonic a swelling. Distilled water naturally represented an extreme hypotonicity. The villi presented some evidence of injury, but regained their normal appearance after some minutes. Two per cent sodium chloride and 10 per cent dextrose were the limits used in the hypertonic series. The sodium chloride was injurious, the dextrose not. Very little difference could be noted in the effect on villi movement of this concentration of dextrose and salt from that observed with isotonic solutions. Distilled water also increased villi movement but not so marked or so persistent as salt and dextrose.

During the study of the action of irritants, observations were made on the rate and rhythm of villi activity. A single villus was picked and watched for a given period of time and the interval elapsing between successive contractions recorded. In the most active fields the average time between successive contractions was about 3 seconds. In sluggish fields the time was so irregular that the term rhythm is scarcely applicable. In sluggish fields one very frequently saw a villus make two or or three contractins at intervals of a few seconds, then rest for a minute or more and then repeat. It is difficult to say what represents a physiological rhythm. One never sees a regularity comparable to that seen in the heart or respiratory mechanism, or seldom even to that of the outer musculature of the intestine.

Small doses of pilocarpin, atropin, nicotin and barium, when applied locally, augmented the activity of the mucosa. Very large doses led to paralysis. The results were similar to those obtained on intravenous administration and will be more fully described under that heading. Pilocarpin when applied locally called forth an abundant secretion of mucus. Nicotin and barium were somewhat irritant. That this irritation was not an important factor is indicated by the fact that it is impossible to introduce enough of the drug intravenously to bring up the concentration to the point necessary for marked irritation on local application.

With the application of epinephrin the intestinal mucosa quickly became anemic. Immediately following this the villi retracted, then remained quiet for about 30 seconds on an average, then began to lengthen and shorten alternately. Usually after the elapse of several minutes the field had the same appearance as at the start. We have kept in mind the observations of various workers that the effect of epinephrin, especially with reference to the type of activity of the vascular system and the outer musculature of the gastro-intestinal system, is dependent upon the concentration of the drug used. We have applied locally concentrations from 1:100 up to 1:6,000,000. In no case where any reaction was noted have we found anything but augmentation of activity, differing only in intensity.

Work on the extrinsic nerves. It is a generally accepted fact that the motor activities of the small intestine depend primarily upon a local intrinsic mechanism. This has been shown in particular with reference to the work on the outer longitudinal and circular musculature. On the other hand, the various types of movement of the outer musculature may be modified either in the direction of augmentation or inhi-

bition by impulse from the central nervous system by way of the vagi and splanchnics.

A series of experiments was carried out to determine if the mucosal motor mechanism is similar in character in this respect. Stimulation of the peripheral ends of both vagi in the vast majority of animals used produced no effect upon the motor activities of the intestinal mucosa. In a few cases a stimulation of the peripheral ends of both vagi resulted in a retraction of the villi and a ridging of the mucosa, but no rhythmical movements were set up. Stimulation was done during quiescence and during activity of various grades and intensity. With the exceptions of the instances mentioned above, no change was wrought in the field under observation. It might be worthy of note that in a number of experiments the outer musculature of the intestine was connected with a recording apparatus and with but few exceptions responded to vagus stimulation with an increase in tonus, rhythmical movement, or both.

In this connection it was thought advisable to determine what effect, if any, decerebration might have. The mucosa was watched in a number of instances during the process. No effect was noticeable.

Attention was next turned to the splanchnics. Stimulation of the peripheral ends of the splanchnics called forth a vigorous reaction on the part of the whole mucosa, contraction of the villi in groups, and a ridging and pitting of the mucosa. No rhythmical movement was set up during stimulation. After cessation of stimulation rhythmical movement in some instances followed, in some not. The reaction was sharp, coming on shortly after the beginning of the stimulation with a tetanizing current. Single shocks were relatively ineffective, producing only a momentary restlessness. A second stimulation thrown in within from 10 to 30 seconds after the first produced no change. This refractory stage in point of duration bore some relation, within certain limits, to the strength of stimulus, the weaker the stimulus the shorter the refractory period.

The reaction of the intestinal mucosa to splanchnic stimulation was sharpest in the duodenum, good in the jejunum, and almost negative in the ileum. In some instances the line of demarcation appeared fairly sharp. In these cases fields were prepared at the ileo-jejunal junction. Stimulation of the splanchnic gave a clear-cut reaction in the upper half of the field and practically none in the lower half. For this work the splanchnics were picked up at the point of emergence from under the diaphragm (pre-ganglionic). Major and minor splanchnics on both sides were stimulated. The mesenteric fibers to the ileum (post-ganglionic) were also picked up and stimulated, the results being negative.

A few animals were used in which no reaction could be obtained through the splanchnic, although the villi presented a normal appearance, were reactive to mechanical stimulation and manifested spontaneous movement. It was thought that perhaps the vagus carried the motor fibers in these cases, but no evidence was found. Epinephrin in these cases was active with one exception. Stimulation of the vagi and splanchnics at the same time gave a typical splanchnic reaction.

Another series of experiments was carried out in which the splanchnics were cut aseptically and time allowed for degeneration. It had been observed in the crucial work that cutting of the splanchnics did not abolish movement of the villi. The nerves were cut in five animals. They were used 4 to 17 days after cutting. The fields of observation in all cases presented a normal appearance and a normal reactivity. Stimulation of the mesenteric fibers (post-ganglionic) provoked a reaction, as did also epinephrin. Stimulation of the vagi after splanchnic cutting gave no results differing from those previously described.

INTRAVENOUS INJECTION OF DRUGS. The drugs chosen were the same series as used in irrigation experiments: epinephrin, pilocarpin, atropin, nicotin and barium.

Reference has already been made to the use of epinephrin in relation to local application and to splanchnic work. It was assumed from the beginning that the effect of this drug upon the motor activity of the intestinal mucosa would be identical with that obtained with splanchnic stimulation. This assumption was borne out in a series of experiments in which the drug was used under a wide variety of conditions. The sharp contraction of the villi, the ridging of the mucosa, the refractory period and the gradual return to the previous state of activity were identical with the splanchnic picture. A number of instances was noted where the response to splanchnic stimulation was feeble before, and very sharp after epinephrin.

In the pilocarpin experiments, the dose was graduated so that the animal received about 0.5 mgm. per kilo body weight. The usual effect on the outer musculature, that of increased tonus and rhythmical contraction, was noted in practically every instance, also a fall in blood pressure and a decrease in heart rate. Synchronous with the decrease in circulation the intestinal mucosa became pale, then gradually recovered its normal appearance with the improvement in circulation. The mucosal motor reaction was very sharp. Synchronous with the reaction of the outer musculature the villi retracted, usually in groups, and the mucosa became grooved and ridged. Unlike the reaction with epineph-

rin, there was no clear-cut period of tonic quiescence but a somewhat rhythmical action of the mucosa and individual villi from the start of the reaction; this usually lasted for a few minutes, gradually diminishing in intensity, and leaving the villi in apparently less reactive condition than normal. Rapid covering of the surface with a thick tenacious and frequently whitish mucus was the rule after pilocarpin. This same phenomenon was commonly observed after nicotin; in fact, after any procedure which called forth a vigorous and sustained reaction, even in cases of repeated splanchnic stimulation or repeated epinephrin injection.

The next series of animals was used to determine primarily the effect of atropin The dosage was graduated so that the animal received about 0.1 mgm. of atropin sulphate intravenously per kilo body weight. Larger and smaller doses were also used. After atropin the villi and submucosa manifested increased activity, but the reaction was not so sharp as with pilocarpin or epinephrin, a minute or two usually elapsing before the activity reached its maximum intensity. The reaction was also more in the direction of a rhythmical one. The outer gut wall was kept under observation during the atropin work. It clearly diminished its tonus and activity after pilocarpin, neither was pilocarpin as effective on the outer musculature after atropin as before. Two points of special interest were repeatedly observed. The effect of vagus stimulation on the intestine was not abolished by doses large enough to eliminate cardiac inhibition, nor was pilocarpin rendered ineffective on the mucosal activity by atropin, except after enormous doses.

Nicotin is a vigorous stimulant to the whole mucosal motor mechanism. The first series of nicotin experiments was carried out to determine the general effect upon the activity of the mucosa. The results were markedly uniform, a sharp contraction of the villi and grooving of the mucosa as a whole, followed usually, but not always, by a short period of quiescence, then rhythmical movement persisting for several minutes. Doses of 1.5 mgm. up to 10.0 mgm were used, depending upon the size of the animal. A second dose gave the same type of response as the first, although not always so sharp. This took place after paralysis of the splanchnic ganglion, this based upon the fact that pre-ganglionic splanchnic stimulation gave no mucosal response. After the initial dose of nicotin the mucosa became covered with a thick whitish mucus. Repeated doses fieally led to a complete loss of activity and reactivity, the paralysis being muscular, inasmuch as no reaction could be obtained with barium.

CORRELATION WITH THE OUTER MUSCULAR MECHANISM. In the course of the experiments noted above we frequently observed vigorous movement of the villi and mucosa as a whole while at the same time the outer musculature of the gut remained quiet. At another time we have observed them both active at the same time. These observations in themselves speak against any close essential interdependence between the mucosa and the outer mechanism, at least so far as motor activity goes. In view of the fact that it is impossible to judge accurately changes in tonicity or to recognize any minute rhythmical movement by direct observation, we conducted a series of experiments where the changes in tonus as well as movement on both the longitudinal and circular muscles were recorded while the villi and mucosa were watched. We were also mindful of the fact that the normal physiological stimulus which sets off the various types of intestinal movements comes from inside of the intestinal canal; consequently attention was also given to any effect which might be produced on the activity of the outer muscles resulting from the mucosal stimulation.

The gut was prepared for mucosal observation as described in the beginning of the paper. In addition a slit was made near one end of the preparation running parallel with the circular muscle and extending almost to the mesenteric attachment. To the lateral edge a few centimeters from the slit a pin was attached to the gut wall which by means of a fine silk cord and a system of pulleys was made to connect with a muscle lever. The pulleys were so mounted that the pull was exerted parallel to the circular muscle. In the same manner a lever was attached to the edge of the slit made, and pull exerted in a direction parallel to the longitudinal muscle. While it is true that the contraction of one group of muscles also influences the position of the lever attached to the other, yet by proper choice of the point of attachment one can reduce this to a negligible amount. In any case there is no difficulty in recognizing the activity of the separate systems of muscles.

Records were taken of the effects of stimulation of the vagi and splanchnics, injection of epinephrin, pilocarpin, atropin, nicotin and barium, and of the application of peppermint oil, and mechanical stimulation of the mucosa.

It was during the course of these experiments that two dogs were found in which the villi contracted upon stimulation of the vagi. Both of these dogs were very reactive, also the outer musculature responded by both an increase in tonus and rhythmical movement, more marked in the circular than in the longitudinal muscle. The response of the outer

musculature to vagus stimulation in almost all of the animals studied was in the same direction, that of increased tonus and rhythmical movement. There was, however, a wide variation in details. In some of these animals there was an initial period of relaxation followed by contraction and in others no relaxation. In fact both of these phenomena were repeatedly observed in the same animal, although so far as could be determined the conditions were the same. There was also considerable difference in the duration of the response. We were not able to pick out and definitely associate any of the details with any activity on the part of the mucosa.

This last statement also applies to the results of splanchnic stimulation. With a few exceptions the splanchnic stimulation resulted in mucosal activity. The effects on the outer musculature were not so constant, both relaxation and contraction appearing, but relaxation predominating. A wide variation in detail was noted. In some instances both muscles ran parallel courses, in others opposite. This was not due to a mechanical defect or to a peculiarity of individual animals, because these variations were observed in the same animal and in the same preparation.

In the dog the reaction of the intestinal mucosa to stimulation cannot be predicted on the basis of the reaction of the outer musculature.

The injection of epinephrin revealed nothing new, the results being essentially those of splanchnic stimulation.

Pilocarpin invariably produced an increased tonicity and frequently rhythmical movement on the part of both longitudinal and circular muscles. On the whole the effect appeared more pronounced on the circular muscle, especially with reference to rhythmical movement. In connection with this it is to be noted that the villi as a rule exhibited rhythmical movement after pilocarpin. At this point we often experienced the phenomenon that although the outer musculature usually reacted in orthodox fashion to a second or third dose of drug, the mucosal mechanism seldom reacted the second time in a fashion at all comparable to the first. The villi became covered with mucus and developed considerable refractiveness to stimulation.

The effect of atropin on the outer musculature noted by us agrees with those usually described, a clear-cut antagonism to pilocarpin without complete stopping of rhythmical movement. The record often revealed an increase in rhythmical movement, if given when the muscles were somewhat relaxed. The mucosal mechanism responded by increased

activity, which however did not come on sharply, but was characterized by a gradual increase. Atropin was not effective after pilocarpin paralysis, but we frequently found pilocarpin active on the mucosal mechanism when stimulation of the vagus, after atropin had been administered, failed to yield any response.

The results with nicotin are rather bewildering. In a number of animals nicotin was ineffective on the outer musculature, although there was a marked vascular and respiratory reaction. In these cases the intestine responded sharply to vagus and splanchnic stimulation and to pilocarpin and epinephrin. The most common effect of nicotin was an increase in tonicity and activity of the outer musculature and a tonic contraction of the villi followed by rhythmical movement. There was, however, no sharp correlation, for we have observed a number of responses of the outer mechanism with none on the part of the inner, and also the reverse.

The effects of barium upon the circular and longitudinal muscles are constant, producing an increase in tonus and rhythmical activity in both, also a contraction of the villi. This might be expected inasmuch as barium acts directly upon the muscles. It was used throughout the work to determine the condition of the muscle in question. It was repeatedly demonstrated that the musculature of the mucosa ceases to respond to barium sooner than the outer musculature of the gut.

During the experiments on the coördination of the mucosal mechanism with the outer musculature, observations were made and recorded on the effect of mechanical and chemical stimulation of the mucosa on the activity of the outer musculature. The results were uniform. In case the outer musculature was relaxed, light mechanical stimulation of the mucosa gave no response, except the local mucosal reaction already described. In case the outer musculature exhibited considerable tonus, even light stroking of the mucosa resulted in quick relaxation more marked on the circular than on the longitudinal muscles. The relaxation persisted during the stroking. The relaxation was usually followed by a return to the normal state and in several instances by an increase in tonus and movement. In case the mucosal stimulation was vigorous, what might be termed punching the gut from the mucosal side, there was an initial loss of tonus quickly followed by an increase in tonus and rhythmical movement (figs. 1, 2, 3 and 4).

VITALITY OF THE MUCOSAL MOTOR MECHANISM. Cannon (7) has pointed out that the myenteric reflex mechanism is relatively long lived, standing up for a considerable time under varying degrees of anemia. It

has not been necessary for us to devise special experiments to determine this point in regard to the mucosal motor mechanism. The rapid collapse of the mechanism under experimental conditions has been to us a constant source of delay and mental irritation.

In planning the experiments on the effect of the drugs mentioned earlier in this paper it was thought that possibly it might be advantageous to employ strips pinned out and immersed in oxygenated isotonic solutions, inasmuch as under these conditions we would not be compelled to contend with radical vascular changes, or changes due to impulses from the central nervous system. This was a fruitless adventure, inasmuch

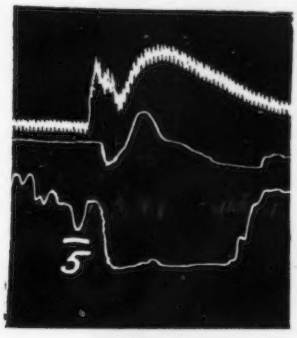

Fig. 1

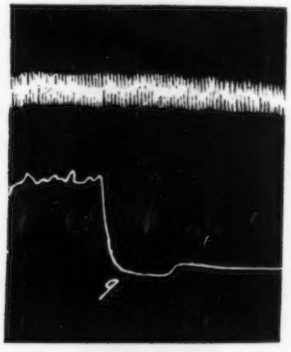

Fig. 2

Fig. 1. Upper curve carotid blood pressure. Middle curve, longitudinal muscle of jejunum. Lower curve, circular muscle of jejunum. *5*. Stimulation of left splanchnic.

Fig. 2. Upper curve carotid blood pressure. Lower curve circular muscle of jejunum. *9*. Light stroking of intestinal mucosa in region of lever attachment.

as we never succeeded in getting any preparation to manifest more than a few feeble villi oscillations. Oxygenated Ringer's, Locke's and Tyrode's solutions were used. We used preparations in which less than a minute elapsed from the time of connection with the living animal and mounting in solution. The outer musculature manifested good activity in practically all cases.

Next a series of perfusion experiments was planned and carried out. Tyrode's fluid, to which had been added about one-third of a volume of defibrinated blood, was used. The pressure bottle was raised so that the pressure at the mouth of the perfusion cannula was approximately

that in the branches of the mesenteric arteries. The perfusion fluid was aerated by passing a stream of air through the fluid in the pressure bottle.

Fig. 3

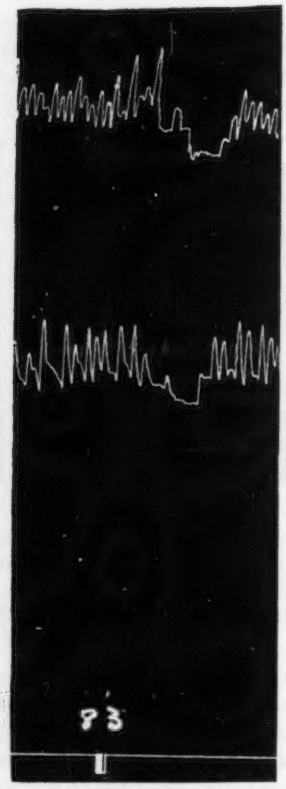

Fig. 4

Fig. 3. Circular muscle of jejunum at levels about 2 cm. apart. At *73* the mucosa was vigorously stroked longitudinally past the three levels.

Fig. 4. Circular muscle of jejunum at levels about 2 cm. apart. At *83* stroking of the mucosa past the point of attachment. Taken from the same preparation as figure 3 but 10 mgm. of nicotin had been injected. Pre-ganglionic splanchnic stimulation not effective.

The fluid passed through a coil of glass tubes in a water bath near the preparation kept at such a temperature that it was delivered at the body temperature of the animal. Having learned from the suspended strip work that shutting off the circulation for only a short time renders the preparation useless, measures were adopted to mount the strip with as little delay and interruption of the circulation as possible. The anastomosis of vessels in the gut wall is good, so that a fair circulation is maintained in a segment a few inches long even though the main vessels supplying that segment be obstructed. A small cannula was inserted into the vessel as rapidly as possible and the perfusion started. The segment was then cut out and mounted while the perfusion was going on.

These preparations were much better than those merely mounted in oxygenated solution, but on the whole were far inferior to those in the intact animal. The villi and submucosa manifested considerable activity, but were relatively refractory to stimulation and short lived The outer musculature on the other hand manifested vigorous activity, readily responding to drugs in an orthodox fashion and retaining its viability for a relatively long time. Epinephrin, pilocarpin, atropin, nicotin and barium were added to the perfusion fluid. The response of the mucosal mechanism in each case corresponded in type to that described under experiments on the intact animal. There is no occasion for going into details relative to these experiments for this reason, and there would be no justification for mention were it not for the fact that the results bore out our previous experience, that the mucosal mechanism is much more sensitive and breaks down under experimental conditions much sooner than the outer mechanism.

Another observation is worthy of note, that of the mucosal motor mechanism in shock. We have already mentioned the fact that villi activity was rarely seen when the animals were under a deep ether anesthesia. This observation led to decerebration as a routine measure. It was our object to decerebrate enough to keep the animal quiet but not to get back far enough to disturb the medulla. A number of our animals early, and a majority in the course of several hours, manifested some degree of shock. In addition to these factors the extensive and frequent handling of the abdominal viscera is a contributing factor in bringing on shock. We observed it frequently. The onset of shock was manifested very early in the intestinal mucosa. The villi became refractory to stimulation, appeared swollen and glistening, became covered with a thick clear mucus, and were congested. These signs appeared often before there was any marked and clear change in blood pressure and respiration.

DISCUSSION

The results of the experiments and observations recorded in the preceding pages of this paper present nothing new so far as the types of movement exhibited by the mucosa of the small intestine are concerned. Many of the details observed, however, are not generally mentioned in discussions on mucosal function, and to our knowledge have not found their way into the literature. The results of the work as a whole suggest more questions than answers. However, the results, when viewed in the proper light, point toward conclusions which serve to emphasize the motor element of the intestinal mucosa as a factor of possibly greater moment than is usually ascribed to it. They may also serve to throw light upon related questions, such as the enteric nervous mechanism, and the activities of the outer musculature of the small intestine.

The morphological basis for the intestinal mucosal motor activity is the muscularis mucosae, which is well developed in all higher animals. It is made up of a thick inner circular and a thinner outer longitudinal layer of muscle. This represents the most common arrangement in the dog, but there is considerable variation. In many cases the layers are of about equal thickness. Again there are frequent interruptions in the coats, the one or the other of the layers being very thin. Again the circular and longitudinal arrangement may not be so clear, the arrangement being retiform in character. From it there extend into the villi muscle strands, running in an axial direction and for the most part attached to the basement membrane of the sides and top of the villus. Trautmann (8) states that the muscular elements of the villi differ in various animals, being best developed in the dog and very poorly so in the cat. This may account, in part at least, for our failure to observe good movement of the villi in our cats. This muscular unit in the villi is innervated directly from Meissner's plexus. Berkley (9) has demonstrated end plates in the main muscularis mucosae and has also followed the fibers to the muscular strands of the villi.

The movements of the villi present some points of interest. One might suppose that, upon shortening, the diameter of the villi would change. There are no circular muscles in the villi of the dog, consequently one would not expect any active ring-like contractions. We have never observed any distinct change in shape or size in the visible part of the villus associated with its activity. This might suggest that the retraction of the villus is not due to any shortening on the part of its body, but that it is merely pulled down from below. This is a difficult point to determine, but if it were the case one would expect regularly to

see some disturbance in the neighboring villi. One commonly, on watching any particular villus, sees it simply slide down and disappear entirely from view without producing the slightest visible disturbance in any of its neighbors. Histological examination of the sections made from active and quiet fields after fixation with Flemming's solution threw no light on the question. Another interesting feature of villi movement is that it is not regularly associated with general mucosal movement. Mucosal ridging, pitting, and group contractions of the villi are rarely seen except after application of some vigorous stimulant.

The application of the term rhythmical to the movement of the individual villi must be made with some qualifications. Rhythm implies regularity of occurrence. In very active fields any single villus usually executes its cycle at somewhat regular intervals, every two or three seconds, but as the field becomes more sluggish the interval becomes more variable and irregular. It often happens that a single villus will complete eight or ten cycles at rather regular intervals and then abruptly stop and remain quiet for many seconds. Another villus adjacent to it will maintain its movement without any variation or disturbance. When one views a large field he is reminded of the incoördinated contractions of the musculature of the heart in delirium cordis, and the temptation arises to apply in case of the villi activity the term delirium villi. This description holds in cases where the mucosa as a whole manifests rhythmical ridging and grooving as well as when quiet. It is suggested on this basis that the impulse which sets off the individual villus does not radiate from a given center but is more sharply localized.

The movement of the mucosa as a whole is more rhythmical than that of the individual villi. There is no progressive wave of movement in the mucosa. It is entirely local in character. So far as we know, Gunn and Underhill are the only observers who have succeeded in recording by mechanical means the movement of the mucosa. The conditions under which they accomplished their work were far from favorable, at least in light of our experience relative to the vitality of the mechanism, so that their results show probably only to a limited degree the possible activities of the mucosa. Their tracings show considerable rhythmicity, but show no evidence of progressive waves of activity, neither do they mention having observed any.

What manner of mechanism is necessary to account for the characteristics of the movements described? Is the stimulus which sets off the movement applied to the muscle through the nerve elements of Meissner's plexus, or to the muscle directly?

It has been demonstrated that the rhythmical movements of the villi and the mucosa as a whole may be set up and augmented by both chemical and mechanical stimulation. In case of mechanical stimulation, if the stimulus is applied to the top of the villus, the reaction which follows does not involve any of the adjacent villi. In case the field is quiet at the time of stimulation, the reaction usually consists of a single contraction of the villus and return, but occasionally the movement persists through a larger number of cycles. In case the field is active, the reaction consists in, first, a quick contraction, then an acceleration of rhythm persisting for a short time. In case the stimulus is applied to the base of the villus, the reaction is in the nature of a group retraction, and in case of persistence of movement, which is more frequent than when the tip is stimulated, each separate villus establishes its own rhythm. In this instance there is no spreading, that is, the movement remains confined to the original site of action. The application of cocain to the mucosa finally abolishes all activity and reactivity, but when this point is reached barium is also ineffective, indicating muscle paralysis.

The results with nicotin, pilocarpin and atropin also bear upon this point. The reaction to the initial dose of nicotin, about 0.5 mgm. per kilo body weight, consists of a sharp retraction of the villi in groups and a ridging and grooving of the mucosa as a whole. This is followed by a few seconds of tonic quiescence, then by rhythmical activity, persisting for some minutes. This dose is usually sufficient to block the effect of pre-ganglionic stimulation. The recovery state finds the mucosa slightly less reactive than normal, but still capable of normal type of response. A second dose provokes a response similar to the first, but less intense. If the nicotin is pushed to the point of disappearance of reactivity, barium is found ineffective again, pointing to muscular paralysis.

Pilocarpin provokes a reaction similar to nicotin, but appears to be more toxic to the mechanism. If a dose be given large enough to give a sharp circulatory reaction, but at the same time allowing prompt recovery, it invariably leaves the mucosa very refractory to stimulation. Barium again at this stage is ineffective.

The reaction to atropin is of a different character. Usually, 15 to 30 seconds elapse, often 1 minute, before there is any noticeable change. The activity of villi closely resembles the normal type seen on first opening the gut. Their activity gradually increases up to a maximum, then gradually diminishes, but the augmentation persists for some minutes.

Pilocarpin is active after atropin, but atropin is not active or is at least less so than normally, after pilocarpin has been given.

The isolated reaction in case of mechanical stimulation of a single villus, the scattered and incoördinated points of activity in a spontaneously active field, and the persistence of activity to the point of muscle paralysis, as evidenced by failure to react to barium, point strongly to a myogenic origin.

Impulses via the vagi nerves as a rule apparently do not reach the intestinal mucosal musculature, in the dog at least. This assertion is based upon the observations that stimulation or cutting both vagi, in the the majority of dogs, does not modify the state of activity or reactivity of the mucosa. The splanchnics carry augmentor impulses. There is no evidence that impulses via the splanchnic directly excite rhythmical activity, the typical reaction being that of tonic contraction followed by some rhythmical movement, this latter coming on after the cessation of stimulation and never during the stimulation. The reactivity of any muscle depends in part upon its degree of tonus. The assumption in this case is that the splanchnic impulses modify the activity of the intestinal mucosa by producing a variation in tonus. So far as we know the distribution of the splanchnic is not essentially different in the ileum, except possibly in the last few inches, from that in the duodenum and jejunum. At least there is not enough difference to account for the differences in response between the duodenum and that of the ileum. The reason for this difference must be sought elsewhere.

Under physiological conditions the main factor which sets off activity of the outer musculature of the intestinal canal is mechanical stimulation by the food mass. Chemical stimulation by substances present in the canal and those absorbed, as well as by so-called peristalic hormones, may also play a part. We have shown that both chemical and mechanical stimulation set the mucosal mechanism into activity. The question then arises: Is there any interrelation and coördination between the activities of the outer-musculature and the mucosa? That there is no essential interdependence is conclusively shown by the data already presented. One cannot predict the state of activity or reactivity of the mucosa by noting the state of the outer musculature. We have repeatedly observed excellent movement of the mucosa and villi during periods of quiescence of one or both of the outer layers of muscle and also during various stages and degrees of activity. Pinching the gut, thus setting up local contractions as well as advancing waves of contraction, does not set up any activity on the part of the mucosa unless the whole gut wall

is picked up and pinched. In this case there is a local reaction in the mucosa such as one sees when stimulating the mucosa directly with a probe or forceps.

Thus it is clear that a stimulus applied to the outer musculature does not affect the mucosa. Does a stimulus to the mucosa affect the outer muscles, and is the reaction of the mucosa in this case definitely coördinated with any phase of the outer muscular activity?

In case the outer musculature is quiet and relaxed, light mechanical stimulation of the mucsoa sufficient to bring about a good reaction on the part of the villi and mucosa produces no immediate effect on the outer musculature. On continued stimulation one sometimes sees a slight increase in tonus and some rhythmical movement. In case the outer musculature is in tonus or manifesting rhythmical movement, stimulation of the mucosa brings on a relaxation. This relaxation persists for some time even though the stimulus be continued, but the outer muscle finally escapes from it. In case of vigorous stimulation there is at first a relaxation of the outer musculature followed by an increase in tonus and also by rhythmical activity similar to that seen on pinching the gut. The main effect seen on stimulation of the mucosa is a relaxation of the outer muscles followed by rapid recovery, which recovery is more rapid in case the stimulation is vigorous. This reaction can be demonstrated in case the outer musculature is thrown into a state of tonus and activity by vagus stimulation, by pilocarpin, and by nicotin, but not after barium. The application of peppermint oil to the surface provokes the same reaction. It is frequently seen on irrigation of the mucosal surface with isotonic dextrose.

What is the explanation of this reaction and its rôle? This throws open the whole question of the enteric motor mechanism. While the data thus far presented do not bear directly upon all phases of the question, yet a few hypotheses would seem to be in order.

It is a generally accepted fact that the motor activities of the outer musculature will go on after section of all extrinsic nerves. Our work shows th s also to be true for the mucosal motor activities. The mechanism in both cases is then inherent in the intestine itself. The main difference between the activities of the outer and the mucosal musculatures lies in the fact that the outer muscles in addition to local rhythmical contractions manifest also a progressive wave of contraction preceded by relaxation, true peristalsis, while the mucosal mechanism manifests only local activities.

Bayliss and Starling (10), Magnus (11) and Cannon (12) consider the peristaltic wave as a reflex dependent upon the plexus of Auerbach. Magnus found that Meissner's plexus takes no part in the so-called reflex. Gaskell is inclined to the view that the peristaltic wave is not a reflex, but is accounted for by the inherent properties of intestinal muscle and that the nervous elements are concerned merely in the regulation of tone.

On the basis of the view that the peristaltic wave is a reflex, it is necessary to assume that the stimulus is carried to the cells of Auerbach's plexus by afferent fibers, causing the cells to discharge impulses which go to the muscles producing contraction just behind the point of excitation and relaxation in front. Dogiel (13) has given evidence that the neurones of Auerbach's plexus have two axons with different terminations thus establishing a possible morphological basis for such a reaction. A survey of the literature would indicate that the reflex theory is the predominating view, as against the view expressed by Gaskell (14).

The tonus function of Auerbach's plexus is not denied by those who hold to the reflex theory; in fact, a reflex tonus mechanism seems necessary to account for the fact that after cutting of the vagi the gastrointestinal tract recovers in time to a large extent its normal tonus.

The peristaltic contractions are abolished by nicotin (15). This is not true for the movement of the mucosal musculature. If the behavior of the peristaltic mechanism to nicotin is an argument for a reflex origin, then it would seem that the mucosal activity is not the same type. This would mean then that Meissner's plexus does not play the rôle of a reflex nervous motor mechanism for the mucosal musculature.

The evidence brought forward in this paper would seem best interpreted in the light that Meissner's plexus relative to the mucosal musculature is a local reflex tonus mechanism. Recent work by Mueller (21) on the development of the vagus and splanchnic in the selachii indicate the presence of afferent fibers in the enteric plexuses of these forms. Accepting the conclusions of Langley (15) that the postganglionic fibers of the autonomic system end directly in the effector organ, and putting with it our observation that local stimulation of the mucosa has the same effect upon both the outer and mucosal musculatures as splanchnic stimulation, the conclusions would be that Meissner's plexus is simply a local mechanism, splanchnic in function.

What then are the physiological relations of Auerbach's and Meissner's plexuses? It is generally conceded that Auerbach's plexus is vagal in origin. The fact that vagus stimulation in the majority of dogs as

well as local stimulation of the outer musculature of the intestine provokes neither contraction nor relaxation in the mucosal musculature would indicate that as a rule no afferent motor or inhibitory impulses pass from Auerbach's plexus to Meissner's or directly to the muscularis mucosae. Meissner's plexus is splanchnic in origin (16). Taking the generally conceded fact that impulses by way of the splanchnic produce relaxation of the outer musculature and our observation of tonic contraction of the muscularis mucosae, we conclude that the splanchnic must carry separate fibers to the outer and mucosal musculatures, or a single fiber must divide, sending one branch to the outer, producing relaxation, and one to the mucosal muscles, producing tonus.

The alternative would be that the splanchnic fibers terminate with and distribute their impulses through the plexus of Meissner. To our knowledge histologists have not furnished clear-cut proof for either hypothesis.

The theory that no ganglionic cells lie in the path of the splanchnic peripheral to the superior mesenteric ganglion is based mainly upon the evidence that after nicotin paralysis of the superior mesenteric ganglion stimulation peripheral to the ganglion provokes a reaction in the intestinal musculature.

The answer to these questions hinges on the effect of nicotin on the enteric ganglia. It is generally conceded that very large doses are required to produce paralysis, much larger than for the autonomic ganglia. If nicotin does not paralyze the ganglia of Meissner's plexus, and our evidence indicates that it does not, then stimulation peripheral to the superior mesenteric ganglion after its paralysis proves nothing as to the termination of the splanchnics.

Magnus has maintained that Meissner's plexus plays no part in the myenteric reflex, but that it is dependent solely upon Auerbach's plexus. This conclusion is based upon the observation that the outer intestinal musculature stripped away from the mucosal, consequently free from Meissner's plexus, manifests on proper stimulation the typical contraction behind and relaxation in front of the point of stimulation. Granting this, may it not be true that in the intact intestine Meissner's plexus does play an important rôle in the motor activities of the intestine during absorption and digestion? We are reminded of the work of Meek (17), who found that on transection of the intestine the peristaltic wave did not pass the point of cutting until the nerve elements had regenerated. Also Cannon (18) found that incisions around the stomach down to the submucosa did not stop the wave. In the light of the data

presented on the reaction of the outer musculature to mucosal stimulation, and knowing that the mucosa is the normal physiological point of stimulation, it is suggested that impulses from the mucosa through Meissner's plexus may have a more important rôle than is usually thought. This does not belittle the importance of gut distension as a stimulus. Further work is now in progress relative to this question.

Our experience with nicotin in relation to the motor activity of the intestinal mucosa would indicate that there is no paralysis in Meissner's plexus, else it is difficult to ascribe any function to it unless it be an afferent mechanism. Nicotin does not paralyze the posterior root ganglia. Our experience with Auerbach's plexus is not large enough to make this statement relative to it, but we are mindful of the fact that the literature on this point is not completely harmonious. Langley himself, who is responsible for the most fruitful work on the autonomic system, using the nicotin procedure, suggests the necessity for further work.

It seems just as plausible, and explains our results better, to assume Meissner's plexus as playing a double rôle, a terminal splanchnic mechanism, and a local reflex mechanism, tonus for the mucosal musculature and inhibitory for the outer. On this basis splanchnic and local stimulation of the mucosa should give identical results. Such is the case.

This necessitates the assumption of afferent elements in Meissner's plexus, which is a mooted question. Gaskell and Langley maintain the absence of such elements. Dogiel (20) maintained cells of his type II to be afferent, and recently Mueller (21) published evidence for such elements in the stomach of selachii.

The differences in activity and reactivity of the mucosa between different levels of the small intestine are very striking. Most of the literature on the activity of the outer musculature makes no mention of the differences, the writers apparently not having noted any difference or attaching no significance to it. Macleod (22) and Lucciani (23) state that there is a difference in peristalsis between the upper and lower ends of the small intestine, peristaltic waves occurring less frequently in the ileum than in the duodenum and jejunum. Alvarez and his co-workers(24),(25) have studied the phenomenon rather extensively and account for it on the basis of a metabolic gradient. They have shown that the gradient curve for the outer musculature runs parallel with that of the intestinal mucosa. The comparatively slight motor activity and low degree of irritability manifested by the villi in the ileum may possibly be accounted for on this basis.

SUMMARY AND CONCLUSIONS

1. The intestinal villi manifest somewhat rhythmical movements, shortening, lengthening and lateral or swaying movement, both singly and in groups.

2. The mucosa as a whole exhibits movements which may be designated as ridging, grooving and pitting.

3. The movements of the individual villi may or may not be associated with movement of the mucosa as a whole.

4. The intestinal mucosal motor mechanism is set into activity by mechanical stimulation, by heat, irritants, and by epinephrin, pilocarpin, atropin, nicotin and barium.

5. The mechanism is very sensitive to anemia and is comparatively short lived.

6. Impulses by way of the vagi usually do not reach the mucosal musculature. The splanchnics carry tonus impulses to the mucosa.

7. Meissner's plexus is looked upon as a terminal splanchnic mechanism and a local reflex tonus mechanism.

8. There is no definite interdependence or correlation between the activities of the outer and mucosal motor mechanisms.

9. Impulses from the mucosa in case the outer musculature is in tonus bring about relaxation. It is suggested that this reaction may play a rôle not hitherto given prominence in considering motor activities of the intestine.

10. The reaction of the intestinal mucosa to stimulation is local in character and myogenic in origin.

11. The mucosal motor mechanism is most active and reactive in the duodenum and upper jejunum, less so in the lower jejunum and almost refractory in the ileum. The metabolic gradient of Alvarez is suggested as the basis for a possible explanation.

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BIBLIOGRAPHY

(1) Lacauchie: Compt. rend. d. l'Acad. franç, 1843, xvi, 1125.
(2) Gruby and Delafond: Ibid., 1843, xvi, 1199.
(3) Bruecke: Sitzungsb. d. K. Akad. der Wissensch., Wien, 1851, vi, 248.
(4) Exner: Pflügers Arch., 1902, lxxxix, 253.
(5) Gunn and Underhill: Quart. Journ. Exper. Phsyiol., 1914, viii, 290.
(6) Hambleton: This Journal, 1914, xxxiv, 446.

(7) Cannon: Ibid., 1913, xxxii, 347.
(8) Trautmann: Anat. Anzeiger, 1909, xxxiv, 113.
(9) Berkley: Ibid., 1893, viii, 12.
(10) Bayliss and Starling: Journ. Physiol., 1899, xxiv, 99.
(11) Magnus: Pflüger's Arch., 1904, cii, 123, 356.
(12) Cannon: This Journal, 1912, xxx, 114.
(13) Dogiel: Arch. Anat. u. Physiol., 1899, 130.
(14) Gaskell: The involuntary nervous system, 1920, 112.
(15) Gaskell: Ibid., 116.
(16) Langley: Schaefer's Textbook of physiology, 1900, ii, 616.
(17) Streeter, Keibel and Mall: Manual of human embryology, 1912, ii, 153.
(18) Meek: This Journal, 1911, xxviii, 356.
(19) Cannon: The mechanical factors of digestion, London, 1911, 193.
(20) Dogiel: Anat. Anzeiger, 1896, xi, 679.
(21) Mueller: Arch. f. mikr. Anat., 1920, xciv, 208.
(22) Macleod: Physiology and biochemistry in modern medicine, 1918, 468.
(23) Luciana: Human physiology (English Trans.), 1913, ii, 237.
(24) Alvarez and Starkweather: This Journal, 1918, xlvii, 67.
(25) Alvarez and Starkweather: Ibid., 1919, l, 252.

A STUDY ON THE PERMEABILITY OF THE PLACENTA

I. Permeability to Agglutinins, Hemolysins, Diphtheria Antitoxin and Diastase

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This investigation was undertaken at the suggestion of Dr. A. J. Carlson in the hope that it might throw some light on the question of the permeability of the placenta to internal secretions.

Thus far direct and indirect experiments in that field have not yielded results which can be readily interpreted. It was decided to attack the problem in this instance by studying the behavior of the placenta toward antibodies and enzymes. These substances can be measured quantitatively in the blood and in the present state of our knowledge it is permissible to assume that antibodies, enzymes and internal secretions are similar. Regardless of the question of similarity, however, such a study shows whether or not the placenta is permeable to any or all of the products elaborated by the various cells and passed into the blood.

Literature. The direct investigation of placental permeability to internal secretions is confined to a few experiments in which a gland of internal secretion has been removed from a pregnant animal at a period in the development of the fetus when the fetal gland was known to be functional.

Carlson (1) found that dogs parathyroidectomized when pregnant developed tetany earlier and more intensely than non-pregnant dogs and that pregnant cats were more depressed. Young born of such animals were normal. Marine (2) and others found that in dogs partial parathyroidectomy results in tetany during pregnancy and lactation, which later disappears if the animal is tided over these periods by the injection of calcium salts, and that the young are normal.

Carlson and his pupils (3), (4) pancreatectomized pregnant dogs near term. The urine of these animals remained sugar-free till the

young were removed from the uterus, after which sugar in quantities typical of pancreatic diabetes appeared. This, however, might mean retention of sugar by the fetus rather than transfer of an internal secretion from fetus to mother, for the dogs were thrown into a condition incompatible with pregnancy and became progressively weaker. On the clinical side the records (5) show that children born of mothers with diabetes mellitus are normal.

Ukitor (6) thyroidectomized rabbits on the 7th and 9th days of gestation. The gestation period was prolonged to 60 or 70 days and the young were born small and weak with incompletely ossified bones and hypertrophied thyroid glands which showed signs of hyperfunctioning. In this laboratory it has been found that the young of dogs and cats with hyperplastic thyroids are born with hyperplastic thyroids.

Lillie (7) concludes that the most plausible assumption to make in explaining the occurrence of the "free-martin" is that the placenta is impermeable to the secretions of the gonads.

On the basis of analyses thus far made it would seem that crystalloids normal to the blood occur in human maternal and fetal blood at the time of delivery in very nearly the same concentrations and tend to vary in the same direction though in the case of some substances constant differences occur. These differences are slight.

Kingsbury and Sedgwick (8) obtained the same average value for uric acid in maternal and fetal bloods in a series of 42 tests. Morriss (9) found the average sugar content of the fetal blood in the 24 cases analyzed to be slightly lower than the maternal. However, the maternal blood sugar rises during the second stage of labor and the values for fetal blood are within the limits normal for adults or higher. Plass (10) and Hunter and Campbell (11) agree that the total creatinine is very nearly the same in maternal and fetal bloods though the average amount is slightly higher in fetal than maternal blood. Urea was found by Slemon and Morriss (12) to be practically the same in the two sera in 16 normal cases and in abnormal cases to vary in the same direction. Morse (13), on the other hand, found that the amino acid content of the fetal blood was materially higher than that of the maternal blood in 13 out of 18 cases, and Edelstein and Ylppo (14) obtained an average value for sodium of 0.032 per cent and for potassium of 0.13 per cent in favor of the fetal blood.

Kito (15) investigated the concentration of diastase in the fetal and maternal sera of guinea pigs under normal condition and when the maternal blood diastase was increased by ligation of the renal

arteries and by uranium acetate poisoning. The fetal blood values varied in members of the same litters and were constantly lower than those of the mothers. An increase in the concentration of diastase in the maternal blood was accompanied by a slight rise in the average diastase content of the fetal bloods. His methods are open to the criticism, however, that the fetuses were bled "from an incision in the neck" and the conditions under which the concentration of diastase in the maternal blood were raised were toxic.

Though the majority of investigators find that antibodies pass the placenta of an actively or passively immunized mother, there is disagreement on the subject.

There are many cases on record of women who have contracted typhoid during pregnancy and later have given birth to normal children either lacking agglutinins in the blood or having a lower concentration agglutinins than the mother. Schumacher (16) compared the titer of agglutinins in blood obtained from the placental side of the cord of infants with the blood of the mother in cases in which the mother gave no history of typhoid. The blood of the infants might be negative when that of the mother was positive and it was always of lower titer than that of the mother but it was never positive when that of the mother was negative. He used the microscopic method but his tests were well controlled. Fellinger and Doell (17) using the macroscopic method made comparative tests for Staphylococcus, B. coli, B. typhosus, and cholera agglutinins, and for bacteriolysins and hemolysins in a series of cases in which the woman gave no history of infection. They found that the titers of the maternal and fetal bloods varied independently. Except in the case of hemolysins, the fetal blood was often of a higher titer than the maternal and might be positive when the maternal blood was negative. They conclude that the normal placenta is impermeable to antibodies and that normally antibodies are found in fetal blood and produced by fetal tissues. This view is supported by Pfaundler (18) and others of his school. Polano (19), who compared the titer of diphtheria antitoxin in fetal and maternal bloods in cases in which the mother gave a history of diphtheria, and of tetanus antitoxin after giving the mother injections of the antitoxin some days previous to delivery, obtained results similar to Schumacher's. He regards this as proof that the placenta is normally more or less permeable to antibodies.

Numerous investigators, among them Polano (20), Halban (21), Langer and Landsteiner (22) agree that the blood of infants rarely contains natural hemolysins.

Römer (23) and Dzierzowski (24) obtained negative results when they immunized horses, cattle, sheep, swine and dogs to tetanus antitoxin, but positive results were obtained by Römer for rabbits, guinea pigs and man. Ehrlich (25) and Ehrlich and Hubner (26) obtained an appreciable degree of passive immunization in the young when they immunized mice to ricin and abrin and guinea pigs to tetanus toxin. Stäubli (27) immunized guinea pigs to formalin-killed cultures of typhoid bacilli actively before and during pregnancy and passively shortly before the birth of the young. The relative concentration of agglutinins in maternal and fetal sera depended upon the length of time the agglutinins had been in the blood of the mother, being equal if the antibody had been present a month or more but progressively lower in the fetal as compared with the maternal serum as the time shortened.

Wegelius (28) performed the same experiments on goats and rabbits using vibriolysin, tetanus toxin and cholera spirilla as antigens. The sera were tested at the birth of the young and for several days following it. The blood of the newborn showed a much higher titer than that of the mother in every case except that of passive immunity conferred shortly before delivery. The subsequent antibody curve of the newborn was one typical of passive immunity. On the contrary Reyman (29) in a study of the natural agglutinins to B. typhosus, B. coli, horse and rabbit sera in a series of 14 goats and their newborn kids found the blood of all but one kid negative. Natural vibriolysins occurred in lower or higher concentration in the blood of the kid, a result also obtained by Schenck (30), but the titer for staphylolysins was lower, a result which agrees with the findings of Polano for man but disagrees with those of Schenck.

Kraus (31) and Kreidl and Mandl (32), who immunized goats to red blood corpuscles, found that the blood of the kid was positive but of a lower titer than that of the mother. The latter investigators injected the fetuses in one series of animals with swine corpuscles and produced an active immunity of the fetus, a passive immunity of the mother. Famulener (33) found no hemolysins in the newborn kids of four goats immunized to sheep's corpuscles.

The discordant results just mentioned are probably due to differences in technique for the conditions of immunization, of the antigen used or of the species of animal, do not account for them.

In this series of experiments the placental permeability of rabbits and guinea pigs to diastase, agglutinins, hemolysins to sheep's corpuscles, and to diphtheria antitoxin has been investigated.

METHODS. *Serum.* In the majority of the experiments the mother animal was killed by a blow over the head, the fetuses were immediately freed from the uterus, and the mother was bled from the heart by means of a syringe. The young were then anesthetized and the blood was drawn from the heart into a small syringe containing glass beads where it was defibrinated. To make sure that no fluid was obtained from the thoracic cavity a hole large enough to expose the heart was made in the body wall. Unless otherwise specified the rabbits were in the 27th to 29th day of the gestation period, the guinea pigs between the 50th and 60th days.

In the few cases in which the sera were tested after the birth of the young, suckling had been prevented by covering the nipples of the mother with collodion.

In all cases the placentas were examined for gross lesions. Nothing which could be interpreted as such was ever found except once and the fetus in that case was dead.

Agglutinins. One series of rabbits was immunized before conception with formalin-killed suspensions of pure strains of B. coli, B. paratyphosus A and B, and of B. typhosus grown on a protein-free medium kindly given me by Mr. C. O. Melick. In this way the results were not confused by a double immunization of the animal to the specific proteins of the bacillus and to the proteins of the culture media from which bacilli grown on culture media containing proteins cannot be entirely freed. All of these rabbits received a single injection of 1 cc. of the antigen about 8 days before term, to stimulate the maternal cells to more active production of antibodies.

For the titration of the serum a 24-hour culture of the same strain of bacillus grown on agar, formalin killed, and made to a concentration of five billion per cubic centimeter was used. Readings are recorded in terms of the actual amount of serum in the last tube showing distinct agglutination. Because of the small amounts of fetal serum available 0.5 cc. of the antigen, 0.5 cc. or less of the serum dilution, and 0.85 per cent salt solution to bring the quantity of the mixture to 1 cc. were used. Maternal and fetal sera were run simultaneously, the antigen being added to the entire series at once just before putting them into the incubator. The tubes were incubated for one hour at 38°C. and read after standing for 24 hours in the refrigerator. Tubes containing the same amounts of serum were read throughout the entire series at the same time for the sake of more accurate comparison.

A second series of rabbits and guinea pigs was passively immunized by the injection of rabbit or guinea pig immune serum, respectively, containing a high titer of agglutinins. The technic was as described above in all other respects.

Hemolysins. Rabbits were immunized before conception by injections of washed sheep's corpuscles and given a final injection of 2 cc. of corpuscles 8 days before term. The technique was as described for agglutinins except that in titrating the sera the dilutions of inactivated serum in amounts of 0.5 cc. or less, 1½ units of fresh guinea pig complement which had been diluted 1:20 with 0.85 per cent salt solution, 0.5 cc. of 2.5 per cent washed sheep's corpuscles, and salt solution to bring the amount to 1½ cc., were used in each tube.

It may be stated that in all of this work the glassware was thoroughly cleaned and dried, a process which usually included sterilization.

Antitoxin. One series of animals was used to test the transfer of diphtheria antitoxin from mother to fetus, a second to test the transfer from fetus to mother.

In the latter series of experiments a laparotomy was performed under light ether anesthesia and the uterus was drawn from the peritoneal cavity. The fetus to be injected was grasped through the wall of the uterus and a hypodermic needle carefully wiped free of serum was run through the uterus and along the vertebral column to the level of the scapula or just beyond it into the peritoneal cavity and ½ to 1½ cc. of serum was slowly injected. Previous experience had shown that serum so injected is very rapidly absorbed. On examination of the fetuses at the end of the period of experiment, 2 to 6 hours, the point of injection was visible as a small red area. In case of large injections small amounts of unabsorbed serum were also found either intramuscularly or in the peritoneal cavity.

An antitoxin of the United States Standard Products Company which contained approximately 1120 units of antitoxin per cubic centimeter was used in these experiments. This serum has been found to produce serum sickness in man very rarely and in no case did it appear to be at all toxic in the animals into which it was injected. The moribund condition of the fetuses of rabbit 201 and guinea pig 101 (table 3) was hardly due to the antitoxin injected. In the case of the former it was almost certainly due to asphyxia caused by an unavoidable delay in freeing the fetuses from the uterus. This may also be true in the latter instance for in several experiments young fetal guinea pigs seen to move in utero

just before the mother was killed were moribund before they were removed from the uterus.

The sera were tested with toxin produced by the same company according to the method described in the *Hygienic Laboratory Bulletin 21*, the fetal serums being pooled. No attempt was made to determine the antitoxin in present quantitatively.

Diastase. A comparison was made of diastase in fetal and maternal sera under normal conditions, when the diastase content of the maternal blood was raised by ligation of the pancreatic duct (34) and when the fetal blood diastase was raised by the injection of fresh rabbit diastase high serum obtained from animals with a ligated duct.

The concentration of diastase in the serum is expressed in terms of the time necessary for a mixture of serum and 0.5 per cent starch solution in the proportion of 1:20 to reach the achroödextrin stage in a water bath at 39°C. A N/2000 iodine solution (IKI) was used as an indicator.

Sera were kept in a freezing mixture in the ice box till tested. Maternal and fetal sera were tested within 1 to 2 hours after having been collected, and at the same time. Samples of maternal blood drawn previous to the experiment were tested at the time they were drawn and again, when possible, with the final maternal and fetal samples. The results were found to check within a few minutes time with previous determinations.

RESULTS AND DISCUSSION

Antibodies. Passive immunity was produced in 20 animals in 16 of which the mother was injected (tables 1 and 2) and in 4 the fetuses (table 3). In two of the latter experiments the mother animal had received an injection of agglutinin high serum some hours before the fetuses were injected. In every case antibodies passed the placenta. In 9 of the 14 experiments with agglutinins including 8 adult and 32 fetal rabbits and 1 adult and 2 fetal guinea pigs the concentrations of agglutinins in the maternal and fetal sera are of the same order. The differences which do occur are relatively slight though appreciable. In 4 of the experiments the titer of the fetal sera is of a lower order than that of the maternal sera. Experiment 11 is of special interest in that the titers of the several sera of the same litter are of different orders.

On the other hand, the concentration of antibodies of maternal and fetal sera in all of the experiments with actively immunized animals

(table 5) are of the same order, the differences which do occur being relatively slight.

The conditions in these two series of experiments are essentially identical except for the fact that the agglutinins are in the circulation but a short time in the first series and it is in that series that maternal and fetal values of different orders occur. This indicates that the ultimate concentrations of antibodies tend to reach very nearly the same levels in maternal and fetal sera, a conclusion also reached by Stäubli (27) in his work on guinea pigs. These experiments also indicate that the rate of transmission of antibodies through the placenta varies for different individuals. The few experiments with guinea pigs (4, 9, 10, table 1) confirm the results obtained by Stäubli who found that agglutinins are very slow to pass the placenta of the guinea pig.

The diphtheria antitoxin passed from maternal to fetal blood (table 2) more slowly than did the agglutinins. On the other hand (table 3), there is a relatively rapid transfer of antitoxin from fetus to mother for though the titer of the maternal blood was low in three instances, the facts that the experiment is of short duration, that time must be allowed for the serum to pass into the fetal blood, and that the quantity of maternal blood is relatively very great, are to be considered. The data do not explain this difference but they suggest that the fetal blood has been overloaded with a foreign protein and that the placenta is acting as an excretory organ. The results might have been different had rabbit or guinea pig serum containing a high concentration of antitoxin been used.

Diastase. The sera of fetuses of the same litter differ from each other and from the maternal serum in diastase content, the differences between maternal and fetal values being most marked in the cat and least marked in the rabbit (tables 5 and 6). In general the concentration in the fetal serum is lower than that of the maternal serum. That this is not due to any inhibitory action on the part of fetal blood was proved by adding fetal sera of low diastase concentration to mixtures of diastase high serum and starch, adding an equal amount of 0.85 per cent salt solution to a second series of tubes as controls.

Ligation of the pancreatic duct, a very simple operation in rabbits involving the minimum amount of trauma, produced marked rise in the diastase content of the maternal blood which is near the maximum within 10 hours after the operation. A corresponding rise does not occur in the fetuses (table 6) but in 2 of the 5 experiments, B 1 and 5,

TABLE 1

The titer of agglutinins in the maternal and fetal sera of animals passively immunized by the injection of serum containing a high titer of agglutinins. The titer is expressed in terms of the least amount of serum producing agglutination of the antigen

NUMBER OF EXPERIMENT	ANIMALS	INJECTION OF IMMUNE SERUM			DURATION OF EXPERIMENT	TITER						REMARKS
		Kind	Titer	Amount		Mother	Fetus I	Fetus II	Fetus III	Fetus IV	Fetus V	
					hours							
1	Rabbit 56	B. coli y		15 cc. intravenously	25	0.004	0.002	0.002	0.004	0.004	0.008	
2	Rabbit 14	B. coli y		10 cc. intravenously 12 cc. intramuscularly	46½	0.025	0.025	0.025	0.025			See tables 3 and 4
3	Rabbit 15	B. coli y		8 cc. intravenously 10 cc. intramuscularly	42	0.015	0.015	0.018	0.018	0.02		
4	Guinea pig 12	B. coli y		4 cc. subcutaneously	22	0.0025	0.05	0.05				
5	Rabbit 34	Para B 12	0.000014	9 cc. intravenously 10 cc. subcutaneously	43	0.00015	0.00015	0.00015				

6	Rabbit 32	Para B 12	0.000014 0.00005	6 cc. intravenously 10 cc. intravenously 8 cc. intravenously	$51\frac{5}{6}$ $42\frac{1}{3}$	0.0005	0.002	0.002	0.006	0.018	0.014	See table 7
7	Rabbit 17	Para B 12	0.000014	?	$34\frac{3}{4}$	0.0002	0.0017	0.002	0.002			See table 3
8	Rabbit 16	Para B 12	0.00005	10 cc. intravenously $8\frac{1}{2}$ cc. intravenously	15	0.0025	0.003	0.003	0.0032	0.0035		See table 7
9	Guinea pig 2	Para B 12	0.000017	? Subcutaneously	$32\frac{1}{2}$	0.01	0.01	0.015				
10	Guinea pig 6	Para B 12	?	12 cc. subcutaneously	21	0.0004	0.004	0.004				
11	Rabbit 201	Para B A188		6 cc. intramuscularly 12 cc. intravenously	25	0.004	0.005	0.005	0.02	0.02	0.03	See table 3
12	Rabbit 55	B. para A188		8 cc. intravenously 12 cc. intramuscularly	28	0.0004	0.0004	0.0002	0.0002	0.0001		
13	Rabbit 76	B. typhosus (Hop)	0.00005	$16\frac{1}{2}$ cc. subcutaneously	23	0.0002	0.0002	0.0002	0.0003	0.0004	0.0005	
14	Rabbit 57	B. typhosus (Hop)	0.00005	10 cc. intravenously	$4\frac{1}{6}$	0.0012	0.0014	0.0014	0.0014	0.0014	0.0016	See table 7

TABLE 2

The determination of diphtheria antitoxin in the blood of fetuses of animals which had received injections of antitoxin

PASSIVE IMMUNIZATION WITH ANTITOXIN				TITRATION OF ANTITOXIN IN MATERNAL AND FETAL SERA					
Animal	Units of antitoxin injected	Hours duration of experiment	Remarks	Grams weight of guinea pigs	Injection of diphtheria toxin and the unknown sera	Hours till death	Reaction	Grams change in weight	Days
				388	1 M.L.D.	31 (days)	Sick several days. Ulceration at point of injection for 23 days	−78	6
Guinea pig 26	1760 (subcutaneously)	24½	Two well developed fetuses. Conditions normal	330	1 M.L.D. + 0.5 cc. fetal serum		None	+102	6
				382	4 M.L.D. + 0.5 cc. fetal serum		Slight discomfort and edema at point of injection	+52	6
				380	28 M.L.D. + 0.5 cc. fetal serum	37	Autopsy typical		
				275	1 M.L.D.	46½	Autopsy typical		
Guinea pig 27	1760 (subcutaneously)	15½	Four well developed fetuses. Conditions normal	305	1 M.L.D. + 0.5 cc. fetal serum	*5 (days)	Slight edema at site of injection		
				290	2 M.L.D. + 0.5 cc. fetal serum		Slight edema at site of injection	+12	4
				310	28 M.L.D. + 0.5 cc. fetal serum	39	Autopsy typical		

Guinea pig 29	588 (subcutaneously)	6	Three fetuses. Conditions normal	258	1 M.L.D.	100	Autopsy typical		
				248	1 M.L.D. + 1 cc. fetal serum		Signs of discomfort two days. Edema at site of injection	+51	6
				255	1 L^+ + 1 cc. fetal serum	21	Autopsy typical		
Guinea pig 30	588 (subcutaneously)	19	Three fetuses. Conditions normal	265	2 M.L.D. + 0.5 cc. maternal serum		None	+72	6
				270	1 M.L.D. + 0.5 cc. fetal serum		Edema at site of injection. Some discomfort	+85	6
				285	28 M.L.D. + 1.0 cc. fetal serum	21½	Autopsy typical		
Rabbit 14 (Belgian hare)	4700 (intravenously)	16½	Eight average sized fetuses. Conditions normal	255	1 M.L.D.	97	Autopsy typical		
				280	1 L^+ + 1 cc. maternal serum		None	− 5	6
				220	1 L^+ + 0.5 cc. maternal serum		None	+10	4
				270	2 L^+ + 0.1 cc. maternal serum	16	Autopsy typical		
				280	1 L^+ + 0.65 cc. fetal serum	24	Autopsy typical		
				300	1 L^+ + 0.35 cc. fetal serum	30	No autopsy		
				259	28 M.L.D. + 0.55 cc. fetal serum	28	Autopsy typical		
				265	1 M.L.D. + 0.95 cc. fetal serum		Slight edema at site of injection	+25	4

* Lobar pneumonia. No signs of the pathological conditions produced by diphtheria toxin.

TABLE 3

The determination of diphtheria antitoxin in the blood of animals the fetuses of which were injected with antitoxin. The antitoxin injected contained approximately 1120 units per cubic centimeter

PASSIVE IMMUNIZATION OF FETUSES WITH DIPHTHERIA ANTITOXIN						TITRATION OF MATERNAL AND FETAL SERA					
Animal	Grams	Days pregnant	Units of antitoxin injected into fetuses	Hours duration of experiment	Remarks	Grams weight of guinea pigs	Injection of diphtheria toxin and the unknown sera	Hours till death	Reaction	Grams change in weight	Days
Rabbit 200	2525	28	3529	10¼	Fetuses abnormally large. Largest dead	300	28 M.L.D. + 2 cc. maternal serum	26	Autopsy typical		
					The other two were normal with normal placentas	270	2 M.L.D. + 2 cc. maternal serum		None	+4	4
						260	1 M.L.D.	102	Autopsy typical		
Rabbit (Belgian hare 17)	2200	23	3000-4000	8½	Fetuses (5) active. Placentas normal	255	2 M.L.D. + 0.7 cc. fetal serum		None	+35	4
						258	2 M.L.D. + 1.0 cc. maternal serum		Slight edema at site of injection	+15	4
						270	28 M.L.D. + 1.0 cc. maternal serum	51.5	Autopsy typical		

Rabbit giant white 201	2730	23–26	4117	6¾	Young (9) in moribund condition but with hearts still beating. Placentas normal	280	1 M.L.D.	92	Autopsy typical		
						310	1 L^+ + 0.5 cc. maternal serum	39	Autopsy typical		
						315	1 L^+ + 1.0 cc. maternal serum	20	Autopsy typical		
						280	28 M.L.D. + 0.5 cc. maternal serum		None	+38	4
						305	4 M.L.D. + 0.5 cc. maternal serum		None	+36	4
Guinea pig 101x		40–50	1763	5½	Young moribund but with beating hearts. Placentas normal	280	2 M.L.D. + 1.0 cc. fetal serum		None	+25	6
						308	2 M.L.D. + 1.0 cc. maternal serum		None	0	6
						320	28 M.L.D. + 1.0 cc. maternal serum	31	Autopsy typical		

TABLE 4.

The titer of agglutinins in the maternal and fetal sera of actively immunized rabbits. The titer is expressed in terms of the least amount of serum producing agglutination or hemolysis

NUMBER OF EXPERIMENT	RABBIT	ANTIGEN	DATE OF LAST INJECTION PREVIOUS TO CONCEPTION	DATE OF LAST INJECTION	DATE OF BIRTH OF YOUNG	TITER OF SERA					
						Mother	Fetus I	Fetus II	Fetus III	Fetus IV	Fetus V
24	14	B. coli y	Never injected			0	0	0	0	0	0
25	27	B. typhosus Hop.	Never injected		Nov. 12	0.12	0.15	0.15	0.15	0.12	0.12
26	30	B. para B 12	Never injected			0	0	0	0	0	0
27	31	B. para B 12	Never injected			0	0	0	0	0	0
*28	20	B. typhosus Hop. (Mercury killed)	Each 5th day throughout gestation			0.015	0.01	0.01			
29	3	Sheep's corpuscles	Nov. 20	Jan. 15	Jan. 24	0.0037	0.0037	0.0037	0.0037	0.0035	
30	6	Sheep's corpuscles	Nov. 20	Jan. 17	Jan. 28	0.0006	0.0006	0.0008	0.0008	0.0008	
31	4	Sheep's corpuscles	Nov. 27	Jan. 31	Feb. 15	0.0022	0.0019	0.0022	0.0035	0.0022	
32	8	Sheep's corpuscles	Nov. 27	Jan. 22	Jan. 29	0.0012	0.0015	0.0012	0.0015	0.0003	0.0012
33	25	B. para B 12	Nov. 30	Jan. 3	Jan. 11	0.014	0.02	0.025	0.037	0.037	0.037
34	7	B. para B 12	Nov. 30	Jan. 3	Jan. 10	0.0012	0.0025	0.0025	0.0025	0.0022	0.0022
35	35	B. para B 12	Feb. 1	Mar. 1	Mar. 9	0.000017	0.000017	0.000017	0.000017	0.000017	
36	26	B. typhosus Hop.	Jan. 23	Feb. 21	Feb. 28	0.0002	0.0002	0.0002	0.0005		
37	10	B. paratyphosus A 188	Jan. 26	Feb. 25	Mar. 6	0.00015	0.00015	0.00015	0.00015	0.00012	0.00012
38	27	B. typhosus Hop.	Jan. 6	Feb. 10	Feb. 18	0.005	0.005	0.005	0.005	0.008	0.008

* By Esther Latoma.

TABLE 5

The diastase content of the sera of maternal and fetal guinea pigs expressed in terms of the time in hours and minutes necessary to digest 0.5 per cent starch paste to the achroödextrin stage. (BV) Blue violet stage, (V) violet stage, (R) red stage, (P) pink stage

ANIMAL	REMARKS	HOURS TO DIGEST STARCH SOLUTION				RATIO OF DIASTASE CONTENT OF MATERNAL TO FETAL BLOOD		
		Maternal	Fetus I	Fetus II	Fetus III	Least	Greatest	Average
Guinea pig I		0:42	1:10					1.66:1
Guinea pig II		0:19	0.26½	0.28		1.38:1	1.46:1	1.43:1
Guinea pig III	Very close to term	0:42	0:42	0:42				1:1
Guinea pig IV	Very close to term	0:23	1.07+(V)	1.07+(BV)		2.91⁺:1		2.91⁺:1
Guinea pig V		1:43	2:08	2:27+(P)	2:27+(V)	1.242:1	1.427⁺:1	
Guinea pig VI	About 40 days pregnant	2:07	3:29	4:08		1.645:1	1:952:1	1.846:1
Guinea pig VII		1:48	3:13+(P)	3:13+(P)	3:13+(P)	1.792:1⁺	1.792⁺:1	1.792⁺:1
Guinea pig VIII	About 55–60 days pregnant	2:28	4:53	4:53+(R)	4:53+(RV)	1.98:1	1.98⁺:1	1.98⁺:1
Guinea pig IX	Very close to term	1:25	1:35	2:15(P)		1.117:1	1.587⁺:1	1.348:1
Guinea pig X	First pregnancy	0:41	1:21	1:09		1.631:1	1.975:1	1.827:1
Guinea pig XO	An old female	0:30	2:45	1:30		3:1	5.5:1	4.25:1
Cat	Fetuses about 75 mm. long	1:03	18:31+(P)	18:51+(P)	18:51+(P)			

TABLE 6

Diastase in the maternal and fetal sera (A) of normal rabbits, (B) of rabbits the concentration of whose blood diastase has been raised by ligation of the pancreatic duct

GROUP	RABBIT	CONDITION OF EXPERIMENT	HOURS DURATION OF EXPERIMENT	REMARKS	TIME FOR DIGESTION OF STARCH SOLUTION									RATIO OF DIASTASE CONTENT OF NORMAL MATERNAL TO FETAL BLOOD		
					Mother											
					Normal	Time of experiment	Fetus I	Fetus II	Fetus III	Fetus IV	Fetus V	Fetus VI	Fetus VII	Least	Greatest	Average
A 1	Belgian hare 76	Normal		Young taken at time of birth	1:36	1:34	2:34	3:36	3:59+ (R)	3:59+ (R)				1.6:1	2.5+:1	2.25+:1
2	Belgian hare 35	Normal		Young taken at time of birth	4:31	4:25	4:11	4:10	5:11+ (P)	5:11+ (R)	5:08			0.951:1	1.17+:1	1.08+:1
3	Belgian hare 34	Normal		Mother sacrificed	2:02	2:07	3:28	4:08						1.55:1	1.9:1	1.79:1
4	55	Normal		Mother sacrificed	1:44	1:43	2:08	2:27	3:15+ (V)					1.05:1	1.34+:1	1.51+:1
5	62	Normal		Mother sacrificed	3:41	3:44	4:16	4:51	4:57	5:02				1.24:1	1.9+:1	1.404:1
6	67	Normal		Mother sacrificed	3:48	5:30	6:18	6:29	7:58					1.4:1	2.09:1	1.7:1
B 1	New Zealand red 101 100	Duct ligated	26	Placentas normal. Fetuses active	3:25	0:10	3:33	3:39	3:40	3:42	3:43	3:43+ (R)		1.03:1	1.08+:1	1.07+:1
2		Duct ligated	24	Placentas normal. Fetuses active	2:05	0:19½	3:35	3:40	4:13	4:16	4:20+ (R)	4:20+ (V)		1.72:1	2.08+:1	1.93+:1
3	Belgian hare 93	Duct ligated	24½	Placentas normal. Fetuses active	3:14	1:33	5:13	4:44	4:28	3:53	3:13	*3:13	*4:23	1:1	1.61:1	1.33:1
4	Belgian hare 42	Duct ligated	27	Placentas normal. Fetuses active	3:07	1:35	2:50	4:30	4:45	4:45+ (R)	5:07	5:22		0.90:1	1:72+:1	1.45+:1
5	New Zealand red 68	Duct ligated	22	Three of the placentas slightly infarcated. Fetuses average sized and active	3:58	0:14	1:06	2:22	2:26	2:30	2:42	2:56	2:59	0.22:1	0.595:1	0.83:1

* Aborted at the beginning of the operation.

(R) red stage, (P) Pink stage, (V) violet stage of digestion.

a comparison of the fetal values with the normal maternal diastase value makes it evident that the diastase concentration of the fetal bloods is definitely higher than is normal. It is difficult to explain this increase on any basis other than that diastase has passed the placenta and accumulated in the fetal blood.

As a result of the injection of the fetuses (table 7) the concentration of diastase in the maternal blood rises in 5 experiments but so does that of 3 of the controls. The concentration of diastase in the blood of the fetuses also rises but not in proportion to the amount of serum absorbed. This is probably because the fetus has a high capacity to destroy or remove any diastase, within limits, beyond the concentration normal to the blood and this capacity is probably the explanation of the negative results, and of the comparatively slight effects when the results are positive, obtained when the concentration of diastase in the maternal blood is increased. Numerous investigators have found that the concentration of blood diastase of the adult animal is very constant and that an injection of diastase produces a very transitory effect. This last is true of newborn rabbits. The concentration of blood diastase was tested in 17 rabbits from 24 to 29 hours after their birth. Six of these rabbits had received injections of rabbit serum high in diastase content 20 hours before the test, the seventh of that litter being used as a control. All of these sera took more than 10 hours, some of them more than 24 hours, to digest the standard starch solution, those from the injected rabbits being no exception.

These experiments show that the placenta of rabbits and guinea pigs is permeable to antibodies and enzymes, and they indicate that the relative concentrations which these substances reach on the two sides of the placenta depends upon whether they are indifferent substances or whether the fetal and maternal organisms possess a mechanism regulating their concentration in the serum. They do not show whether internal secretions, if that is what we are dealing with in the case of the parathyroids and pancreas, do or do not pass the placenta. It may be that in the case of insufficiency of these secretions their concentrations in the fetal blood is below the threshold value to which the placenta is permeable or it may be that the placental actually acts as a protective mechanism.

(R) red stage, (P) Pink stage, (V) violet stage of digestion.

TABLE 7

Group A. The concentration of blood diastase of rabbits before and after laparotomy and trauma.
Group B. The concentration of blood diastase of rabbits before and after the injection of the fetuses with rabbit diastase high serum. The titer of the serum is expressed in terms of the time necessary to digest the standard starch solution

GROUP	RABBIT	CONDITION OF EXPERIMENT	HOURS DURATION OF EXPERIMENT	REMARKS	TIME FOR DIGESTION OF STARCH SOLUTION								RATIO OF DIASTASE CONTENT OF NORMAL MATERNAL AND OF FETAL SERA		
					Mother			Fetus I	Fetus II	Fetus III	Fetus IV	Fetus V	Fetus VI			
					Before	After	Difference							Least	Greatest	Average
A 1	10	Laparotomy and trauma	3	The blood diastase had been very constant from day to day	5:00	5:44	−0:44									
2	205	Laparotomy and trauma	2½	Pregnant	1:24	1:25	0									
3	210	Laparotomy and trauma	3½		6:08	6:08	0									
4	206	Laparotomy and trauma	2¾	Blood diastase varied slightly from day to day	5:40	3:40	+2:00									
5	211	Laparotomy and trauma	3	Blood diastase very constant from day to day	5:36	3:28	+2:98									
6	215	Laparotomy and trauma	3½	Blood diastase very constant from day to day	6:24	5:08	+1:16									

		FETUSES INJECTED WITH SERUM			CONDITIONS AT END OF EXPERIMENT											
		Titer	Amount													
B 1	203	19 min.	8½ cc.	2⅛	Placentas normal. Fetuses active. IV and V relatively underdeveloped	3:06	2:02	+1:04	1:54	2:02	4:58	6:38	6:38	Lost	2.51:1	2.3:1	0.62:1
2	204	19 min.	4½ cc.	2⅛	Placentas normal. Young active	2:50	2:50	0	2:30	2:52	2:58				0.78:1	1.14:1	0.95:1
3	57	10 min.	6 cc.	3	Placentas normal. Young active	6:25	3:23	+3:03	6:05	6:23	6:25	6:53	*6:53+ (V)	6:53+ (RV)	1.31:1	1.15:1	1.0:1
4	60	14 min.	4½ cc.	3	Placentas normal. Young active	5:02	4:09	+0.53	4:46	4:19					0.85:1		0.90:1
5	32	10 min.	9 cc.	4:10	Placentas normal. Young active	4:27	2:48	+1:39	3:29	3:39	5:29	5:30	y5:16	y5,30+ (RV)	0.79:1	1.23:1	1.02:1
6	16	14 min.	5 cc.	3	Placentas normal. Fetuses becoming moribund	3:52	2:57	+1:55	3:46	4:20	4:38				0.96:1	1.2:0	1.1:1

* Not injected.
y Aborted when the animal was put under ether.
(V) violet stage, (RV) red violet stage of digestion.

TABLE 7

Group A. The concentration of blood diastase of rabbits before and after laparotomy and trauma

Group B. The concentration of blood diastase of rabbits before and after the injection of the fetuses with rabbit diastase high serum. The titer of the serum is expressed in terms of the time necessary to digest the standard starch solution

GROUP	RABBIT	CONDITION OF EXPERIMENT	HOURS DURATION OF EXPERIMENT	REMARKS	TIME FOR DIGESTION OF STARCH SOLUTION								RATIO OF DIASTASE CONTENT OF NORMAL MATERNAL AND OF FETAL SERA		
					Mother			Fetus I	Fetus II	Fetus III	Fetus IV	Fetus V	Fetus VI			
					Before	After	Difference							Least	Greatest	Average
A 1	10	Laparotomy and trauma	3	The blood diastase had been very constant from day to day	5:00	5:44	−0:44									
2	205	Laparotomy and trauma	2½	Pregnant	1:24	1:25	0									
3	210	Laparotomy and trauma	3½		6:08	6:08	0									
4	206	Laparotomy and trauma	2¾	Blood diastase varied slightly from day to day	5:40	3:40	+2:00									
5	211	Laparotomy and trauma	3	Blood diastase very constant from day to day	5:36	3:28	+2:98									
6	215	Laparotomy and trauma	3½	Blood diastase very constant from day to day	6:24	5:08	+1:16									

		FETUSES INJECTED WITH SERUM			CONDITIONS AT END OF EXPERIMENT											
		Titer	Amount													
B 1	203	19 min.	8½ cc.	2⅛	Placentas normal. Fetuses active. IV and V relatively underdeveloped	3:06	2:02	+1:04	1:54	2:02	4:58	6:38	6:38	Lost	2.51:1	2.3:1	0.62:1
2	204	19 min.	4½ cc.	2⅛	Placentas normal. Young active	2:50	2:50	0	2:30	2:52	2:58				0.78:1	1.14:1	0.95:1
3	57	10 min.	6 cc.	3	Placentas normal. Young active	6:25	3:23	+3:03	6:05	6:23	6:25	6:53	*6:53+ (V)	6:53+ (RV)	1.31:1	1.15:1	1.0:1
4	60	14 min.	4½ cc.	3	Placentas normal. Young active	5:02	4:09	+0.53	4:46	4:19					0.85:1		0.90:1
5	32	10 min.	9 cc.	4:10	Placentas normal. Young active	4:27	2:48	+1:39	3:29	3:39	5:29	5:30	y5:16	y5,30+ (RV)	0.79:1	1.23:1	1.02:1
6	16	14 min.	5 cc.	3	Placentas normal. Fetuses becoming moribund	3:52	2:57	+1:55	3:46	4:20	4:38				0.96:1	1.2:0	1.1:1

* Not injected.
y Aborted when the animal was put under ether.
(V) violet stage, (RV) red violet stage of digestion.

SUMMARY

1. The permeability of the placenta of rabbits and of guinea pigs to agglutinins, hemolysin to sheep's corpuscles, diphtheria antitoxin, and diastase has been studied.

2. Agglutinins and hemolysins tend to come to essentially the same concentration in the maternal and fetal blood, the difference between the two being relatively slight.

3. Diphtheria antitoxin passes rapidly from fetus to mother, slowly from mother to fetus The antitoxin used was a highly concentrated commercial product which may possibly account for the difference between these results and those obtained with agglutinins and hemolysins.

4. Normally the concentration of diastase in the fetal blood is lower than that of the maternal blood. An increase in the concentration of the maternal blood diastase is followed by a relatively slight increase in the diastase content of the fetal sera in some cases. The negative results and the low fetal values when the results are positive are probably accounted for by a great capacity on the part of the fetus to destroy or remove from the blood diastase in excess of the concentration normal to the blood.

I wish to express to Dr. A. J. Carlson and to Mr. C. O. Melick my sincere appreciation of their interest in this work and of their many helpful suggestions.

BIBLIOGRAPHY

(1) Carlson: Proc. Soc. Exper. Biol., 1912–13, x, 183.
(2) Marine: Journ. Exper. Med., 1914, xix, 89.
(3) Carlson and Drennan: This Journal, 1911, xxxviii, 391.
(4) Carlson, Orr and Jones: Journ. Biol. Chem., 1914, xvii, 19.
(5) Joslin: Boston Med. and Surg. Journ., 1915, clxxiii, 841.
(6) Ukito: Oct. Schol. Med. Kyoto, iii, 287.
(7) Lillie: Journ. Exper. Zoöl., 1917, xxiii, 371.
(8) Kingsbury and Sedgwick; Journ. Biol. Chem., 1917, xxxi, 261.
(9) Morriss: Johns Hopkins Hosp. Bull., 1917, xxviii, 141.
(10) Plass: Johns Hopkins Bull., 1917, xxviii, 137.
(11) Hunter, and Campbell: Journ. Biol. Chem., 1918, xxxiv, 5.
(12) Slemons and Morriss: Bull. Johns Hopkins, Hosp., 1916, xxvii, 343.
(13) Morse: Johns Hopkins Hosp. Bull., 1917, xxviii, 199.
(14) Edelstein and Ylppo: Zeitschr. f. Kinderheilk., 1920, xxvii, 79.
(15) Kito: This Journal, 1919, xlviii, 481.
(16) Schumacher: Zeitschr. f. Hygiene, 1901, xxxvii, 232.

(17) Fellinberger and Doell: Zeitschr. f. Geburtsh. u. Gynaekol., 1913, lvii, 285.
(18) Pfaundler: Arch. f. Kinderheilk. 1907, xlvii.
(19) Polano: Zeitschr. f. Geburtsh. u. Gynaekol., 1904, liii, 456.
(20) Polano: Habilitationschrift, Wurzburg, 1904.
(21) Halban: Münch. Med. Wochenschr., 1902, xlix, 473.
(22) Langer and Landsteiner: Zeitschr. f. Heilk., 1903.
(23) Römer: Beitr. z. exper. Therap. 1905, ix.
(24) Dzierzgowski: Centralbl. f. Allg. Pathol. u. path. Anat., 1901, xii.
(25) Ehrlich: Zeitschr. f. Hygiene, 1892, xii, 183.
(26) Ehrlich and Hübener: Ibid., 1894, xviii, 51.
(27) Stäubli: Centralbl. f. Bakt. u. Parasitenk. Orig., 1903, xxxii, 458.
(28) Wegelius: Arch. f. Gyn., 1911, xciv, 18.
(29) Reyman: Journ. Immunol., 1920, v, 227, 391, 455.
(30) Schenck: Monatschr. f. Geburtsh. u. Gynaekol., 1904, 17.
(31) Kraus: Wien. klin. Wochenschr., 1901, xiv, 737.
(32) Kreidl und Mandl: Ibid., 1904, xvii, 611.
(33) Famulener: Journ. Inf. Dis., 1912, x, 332.
(34) Gould and Carlson: This Journal, 1911, xxix, 165.

URINE FORMATION IN THE PERFUSED KIDNEY

The Influence of Alterations in Renal Blood Pressure on the Amount and Composition of Urine[1]

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Two views have, as is well known, formed the center of discussion concerning the nature of the process by which fluid is separated from the blood as it passes through the capillaries of the glomerulus: the filtration theory of Ludwig and the secretion theory of Heidenhain. The evidence and arguments for and against these views are well known and have been recently subjected to an admirable critical review (1).

Before describing the experiments which form the subject of this paper, it is desirable to restate certain facts in connection with the origin of these theories. Ludwig's conception developed from study of the anatomical arrangement of the blood vessels of the kidney and from the principles of hydraulics. Among the experiments subsequently made in Ludwig's laboratory from which issued its greatest support are those of Goll (2), Hermann (3) and Ustimowitsch (4), which demonstrated the parallelism between renal blood pressure and urine elimination. Hermann's experiments are particularly significant because in the analysis of his results one of the chief points of subsequent issue between Ludwig and Heidenhain was recognized and discussed. Hermann applied an adjustable clamp to the renal artery and showed that urine elimination decreased or increased when the renal artery was narrowed or reopened. He regarded these changes in urine as the results of the changes in pressure, though he clearly recognized that both pressure and velocity of blood flow in the renal circulation were altered by his procedures. In discussing the results he says (5):

[1] The experiments described in this paper were reported at the twenty-ninth annual meeting of the American Physiological Society (This Journal, 1917, xlii, 592). The hirudin used in these experiments was obtained through the generosity of Mr. R. A. F. Penrose, to whom grateful acknowledgment is made.

Da aber durch unser Verfahren Geschwindigkeit und Spannung des Blutstromes Abnahme erleiden, so müssen die Erscheinungen hinsichtlich der einen oder andern Veränderung genauer analysirt werden.

Die Geschwindigkeit des Blutstromes kann nur in Betracht kommen hinsichtlich der Quantität des Blutes, welche dem absondernden Organ zugeführt wird; soll nun die Wirkung der Verengerung auf die veränderte Geschwindigkeit des Blutstromes bezogen werden, so muss man noch den Zusatz machen, das in der Niere Kräfte vorhanden seien, welche den Übergang der Harnbestandtheile in die Canälchen vermitteln. Dies vorausgesetzt, muss die Harnabscheidung so lange dauern, als überhaupt noch Blut durch die Gefässe der Niere strömt. Es wurde aber durch den Versuch am lebenden Thiere festgestellt, das nicht nur die Abnahme der Harnsecretion in keinem Verhältniss steht zur Abnahme der Geschwindigkeit des Blutstromes, sondern dass auch die Harnausscheidung ganz aufhörte während noch ein ziemlich rascher Blutstrom durch die Niere ging. Ebenso wurde die Harnausscheidung aufgehoben, wenn die Spannung in den Harncanälchen ein gewisses Maximum erreichte; auch in diesen Fällen war die Stromschnelle des Blutes noch ziemlich bedeutend. Aus diesen Hinweisungen ergibt sich offenbar, das die Geschwindigkeit des Blutstromes in keinen ursächlichen Zusammenhang zu bringen ist mit den Anderungen der Harnabsonderung.

Die Harnabsonderung muss somit also eine Function des Blutdruckes betrachtet werden. . . . ; wenn Blut unter niedriger Spannung die Niere durchströmt, und dennoch keine Absonderung erfolgt, so sind alle anderen Bedingungen vorhanden, ausgenommen die frühere Spannungshöhe, denn die Secretion erfolgt unmittelbar, wenn der Blutstrom eine höhere Spannung wieder annimmt.

This clearly states the position taken by Ludwig's school on the question of the relative importance of blood pressure and blood flow in the glomerular formation of urine.

Heidenhain's denial of the filtration hypothesis originated in the first instance from his observations on the elimination of sodium sulphindigotate (6). When injected into the blood stream of rabbits after the spinal cord had been cut, its presence was demonstrated in the lumen and cells of the tubules but not in the capsule. The elaboration of this observation led Heidenhain to support Bowman's conception of secretion by the tubules and it seems certain that it directed his thought toward the question of secretion rather than filtration in the glomerulus. The chief question concerning the nature of glomerular function on which he differed from Ludwig involved the interpretation of results of venous obstruction. It had long been known that occlusion of the renal vein caused cessation of urine. Ludwig, however, demonstrated that complete venous occlusion caused such engorgement of veins in the boundary zone that the tubules were compressed and their lumina obliterated. He further showed that ureter flow from a kidney perfused with gum solution stopped if the renal vein was ligated. Ludwig therefore could see no insuperable argument

against the filtration hypothesis in the failure of venous obstruction to increase urine flow. Heidenhain, however, accepting Ludwig's view of the end results of complete venous occlusion, believed that before the complete development of vascular engorgement, as pictured by Ludwig, an increase in glomerular pressure should occur, and that this should therefore be associated with transitory increase in urine (7). Later experiments by Paneth (8) in Heidenhain's laboratory showed further that Ludwig's explanation did not cover the phenomenon of cessation of urine from partial venous obstruction, for the injection of a diuretic salt under these conditions caused resumption of urine flow. According to the Ludwig view, any rise of glomerular pressure, however caused, should cause increase of urine: venous obstruction, according to Heidenhain, must cause rise of glomerular pressure: it decreases urine. "Diese Thatsache steht, soweit ich sehe, in schroffstem Widerstand mit der Druckhypothese" (9). Since velocity of blood flow is increased when glomerular pressure is increased from the arterial side but diminished when glomerular pressure is increased by venous obstruction, urine elimination, he concluded, is determined by velocity of blood flow rather than by glomerular pressure. The corollary of this conclusion was that urine is formed in the glomerulus by the secretory activity of the epithelium, the function of which is altered by changes in the blood flow through the capillaries which it envelops.

In the mass of evidence which has accumulated since Heidenhain's publication there is, so far as we know, nothing which enables one to arrive at a clear decision as to the relative importance of these two factors, blood pressure and blood flow in the glomerulus. It is true that attention has been called to the fact that stagnation of blood in the glomerular capillaries incident to venous obstruction would result in an effective increase in osmotic resistance to filtration (10), but it has not been shown that this factor could more than neutralize the effects of increased glomerular pressure in a condition of partial venous obstruction. The support which the filtration theory has received since the time of Heidenhain and which has resulted in its more general acceptance in recent years has come from other directions than that of analysis of the importance of glomerular blood flow and glomerular blood pressure. If the two factors could be dissociated, one being kept constant while the other is varied, a decisive answer might be obtained to this question—one of the oldest in the history of physiological investigation of the kidney.

In a previous paper (11) we published the results of experiments in which the action of caffeine was tested upon the rabbit's kidney perfused *in situ* with hirudinized blood by means of the apparatus designed by Richards and Drinker (12) under conditions which enabled maintenance of constancy of blood flow through the organ, regardless of changes in the caliber of the renal vessels. As an outcome we were able to say that the diuretic effect of caffeine is manifest even when the velocity of the blood current does not increase and we thought that the old idea of renal cell stimulation by caffeine received indirect support from this observation.

In making these experiments with caffeine it became obvious that the method employed would be useful in attempts to study alteration in renal blood pressure in the absence of the associated effects on blood flow which are inevitable in the intact animal; it was also apparent that such data were necessary for the interpretation of effects brought about by the introduction of diuretics. We therefore carried through the experiments herein described in order to learn whether rise in renal blood pressure, under conditions which prevented significant change in blood flow, would cause an increase in urine formation.

Since circumstances have delayed the publication of these results for years since the publication of our first paper, a brief description of the methods used will be given, though they were similar to those previously described.

METHODS. For each experiment two rabbits were used. Each received 2 grams of urethane per kilo by stomach tube. Ether was used during the operation when necessary to maintain proper anesthesia. One rabbit was used as the subject of the experiment; the other was used to supply blood for the perfusion. Usually both had been kept on a liberal diet of carrots for some time before the experiment. The operation on the rabbit used as subject of the experiment was as follows. Cannulae were inserted, one into the trachea for artificial respiration, one into each carotid artery (for record of blood pressure and for supplying blood to the perfusion pump respectively), and one into an external jugular vein for injections. The gastrointestinal tract was removed without loss of blood after successive ligation of coeliac axis, superior mesenteric and inferior mesenteric arteries and portal vein. The vessels of the right kidney were ligated. In some of the experiments the splanchnic nerves on both sides were divided.

The abdominal aorta was ligated about an inch below the origin of the left renal artery, and lumbar arteries issuing between the level of the renal artery and the ligature were tied. The inferior vena cava was similarly ligated at the same level and, after clamping, a cannula was inserted into it and another into the aorta pointing toward the heart. These cannulae were connected with each other and with the output tube of the perfusion pump by means of a small 4-way glass tube, the fourth arm of which served for the introduction of the bulb of a small thermometer into the perfusion stream. By this means blood could be driven from the pump either into the cava or into the aorta.

A loose ligature was laid about the abdominal aorta proximal to the origin of the left renal artery.

Two lumbar arteries are commonly given off from the aorta between the coeliac axis and the left renal artery. We did not attempt to ligature these. The position of the ligature on the aorta determined whether these vessels were included with the kidney in the perfusion system. A sketch is included in each chart showing where the ligature was placed.

When the operation on the first rabbit was finished, the other rabbit was bled from the abdominal aorta; 12 to 15 cc. of blood were collected in a dish containing 130 to 150 mgm. of hirudin. After thorough stirring, it was filtered through cotton and slowly injected into the vein of the first rabbit. In some experiments an equal amount of blood was then taken from the first. The rest of the blood of the second rabbit was mixed with 70 mgm. of hirudin, filtered and used for filling the pump and its connections. The blood from a third rabbit was sometimes needed as a reserve supply.

Having then a rabbit prepared for perfusion whose blood was incoagulable and having filled the pump with hirudinized blood, the reservoir which constituted the intake of the pump was connected with the animal's carotid artery. The outlet of the pump was connected with the two cannulae, one in the inferior cava, the other in the abdominal aorta. With the clamp on the cava loosened the pump was started and at the same time a clamp on the carotid opened to such an extent as was necessary to keep the level of blood in the pump reservoir constant. The animal was then bleeding into the pump at the same rate as that at which the pump was injecting blood into the animal's body; in this way the blood in the pump and connections was thoroughly mixed with that in the animal's body. At a given signal the clamp on the cava was closed, that on the aorta opened, and the

ligature on the aorta above the left renal artery tied tightly. Then the pump was driving blood, received from the animal's carotid, through the vessels of the left kidney via the aorta; at no time was the circulation through the kidney interrupted. In some instances no interruption in urine flow occurred when the artificial circulation was substituted for the natural.

Hürthle membrane manometers were used to register the carotid pressure of the animal and the perfusion pressure from the pump. Urine flow was measured by noting the position of the meniscus of urine in a long graduated cannula (2- or 4-cc., graduated in $\frac{1}{10}$'s) tied into the left ureter, and held nearly horizontally. Blood flow through the kidney was measured by temporarily diverting the blood from the carotid into an alternate reservoir and then recording the time required for the pump to lower the level of blood in the reservoir from one graduation to another etched on the reservoir.

A diagram of the arrangement of the perfusion system is shown in figure 1.

Urine samples, obtained throughout the course of an experiment, were combined in such quantities as the necessities of the methods demanded and were analyzed according to the following methods: Chlorides according to Volhard (1–2 cc. urine; $\frac{N}{10}$ $AgNO_3$; $\frac{N}{20}$ KSCN); urea according to Van Slyke and Cullen (13) or Folin (14); creatinine according to Folin (15). In some experiments, blood used for perfusion was analyzed; for urea according to Van Slyke and Cullen (13); chlorides after fusion with Na_2O_2 according to Volhard. The chief purpose of these analyses was to learn whether the fluid obtained from the ureter was urine, i.e., whether, under the artificial conditions described, the kidney retained its power of elaborating a fluid of characteristically different composition from that of blood plasma.

The means used for raising pressure in the renal circulation were these: Stimulation of left splanchnic nerve; injection of adrenalin;[2] partial obstruction of the renal vein. In one experiment nitroglycerine was used and in others spontaneous changes in the condition of the animal occurred which resulted in constriction of the renal vessels. It should be remembered that the pump has so much excess power in its stroke that its delivery is not affected by changes in the caliber of the renal vessels.

[2] The 1:1000 solution made by Parke, Davis & Co., was used in these and in the experiments described in the two following papers.

RESULTS. The results of seven experiments carried out according to these methods will be presented in the form of charts, amplified by such portions of the original records as are necessary to further illus-

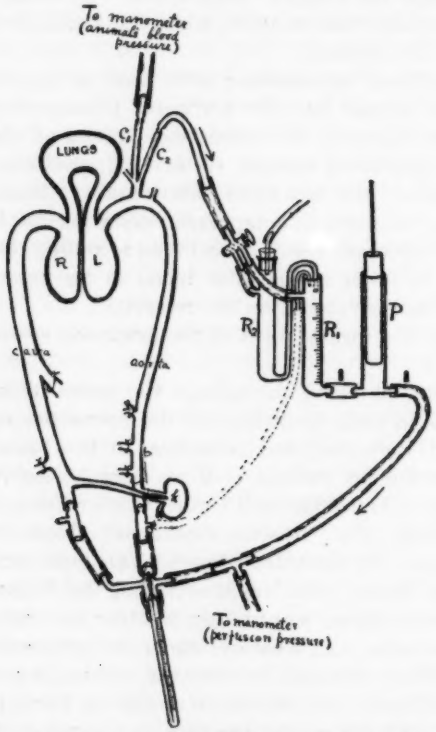


Fig. 1. Diagram of perfusion pump and its connections with the blood vessels: c_1, right carotid artery, connected with Hürthle manometer; c_2, left carotid artery connected with intake reservoirs, R_1, R_2, of pump, P. The dotted lines indicate a rubber tube from the perfusion cannula to intake reservoir by which blood was circulated within the pump system before beginning perfusion; by this means all of the blood in the perfusion system was set in motion and brought to proper temperature before sending it into the renal vessels.

trate points of especial interest. The horizontal lines in the charts which constitute the records of urine flow, carotid pressure and perfusion pressure represent periods of irregular length. It was impossible to make readings of the urine column at regularly uniform inter-

vals. In making the charts the length of periods was dictated by the intervals between urine readings. In the earlier experiments this results in slight distortions of the curve. In later experiments the periods were short, so that this statement is less applicable.

Experiment I. October 5, 1915. (Charts 1 and 2.) In this experiment the pressure in the renal circulation was altered by changes in the thrust and rate of the pump. It therefore does not conform to the demands of the problem as outlined, but rather imitates the circulatory changes in the kidney of the intact animal when a rise in general blood pressure occurs without alteration in the caliber of the renal blood vessels. It may, therefore, serve as a control for the others in which similar changes in pressure occurred without changes in blood flow. The record shows parallelism of renal blood flow (delivery of pump), perfusion pressure and urine output. The parallelism is stricter between perfusion pressure and urine than between blood flow and urine, but there is nothing to show clearly that urine flow depends on the one more than the other. In this respect the difficulties encountered in experiments with intact animals are duplicated.

Analysis of urines of this experiment (chart 2) reveals the important fact that the kidney, perfused under these conditions, retains its power of elaborating *urine* from the blood.

The bladder urine of this rabbit contained chlorides in concentration equal to 0.346 mgm. NaCl per cc. Chloride concentration in the blood at the end of the experiment was 5.98 mgm. per cc. The kidney was preventing elimination of chlorides. Evisceration and hirudin injection caused diuresis and interfered with this power to such an extent that chloride content rose to 4.49 mgm. NaCl per cc. During the first perfusion period (period IV, low pressure, low rate of urine flow) the figure for chlorides sank to 4.1. As a matter of fact it must have fallen to a lower figure than this. The pelvis of the rabbit's kidney is able to hold about 0.6 to 0.8 cc. of urine at little above zero pressure. Of the 1.9 cc. collected in period IV, possibly one-third may have consisted of urine of the previous period, with a concentration of not less than 4.49 mgm. per cc. Hence the remaining two-thirds of period IV must have been as low as or lower than 3.9 mgm. NaCl per cc. During period V (high presure, diuresis) chlorides rose to 5.6 mgm. per cc., but again in VII, with low pressure and low rate of urine flow went down to 3.4 or lower. The point to be emphasized is that the kidney, under perfusion, recovered its power of retaining chlorides, temporarily masked by events earlier in the experiment.

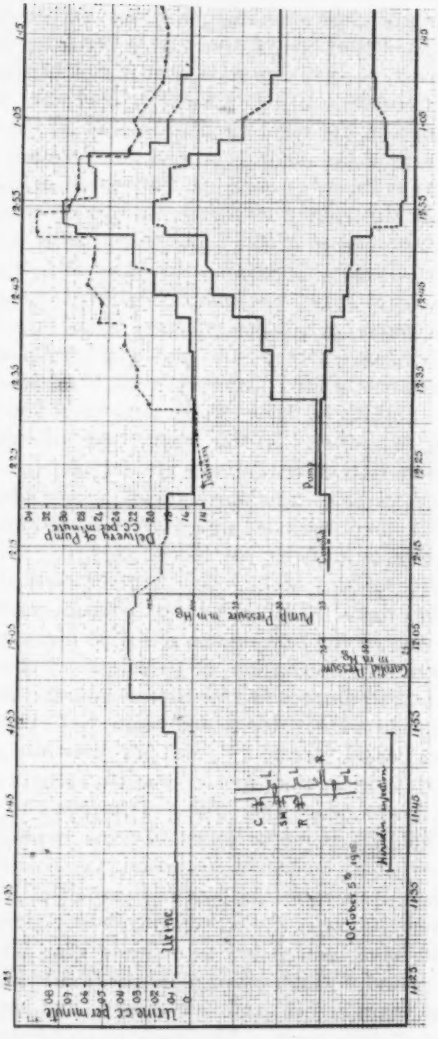


Chart 1 (left half)

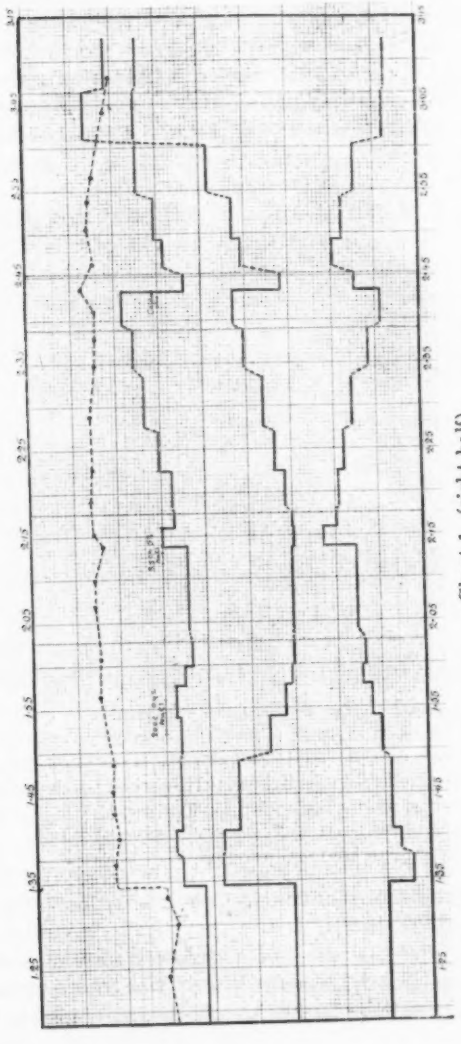


Chart 1 (right half)

Chart 1. Experiment I, October 5, 1915. Perfusion of kidney at varying rates of blood flow and pressure. Splanchnic nerves not cut.

Figures for urea and creatinine are, as was to be expected, less striking than for chlorides. They show a steady diminution from 1.125 mgm. urea N per cc. in bladder urine to 0.35 in the last sample. Urea N in the blood at the end of the experiment was 0.126 mgm. per cc. Creatinine in urine similarly diminished from 0.5 and 0.55 mgm. per cc. to 0.13. Creatinine in blood was not estimated. Folin and Denis (16) give the concentration of creatinine in rabbit's blood as 0.01 mgm.

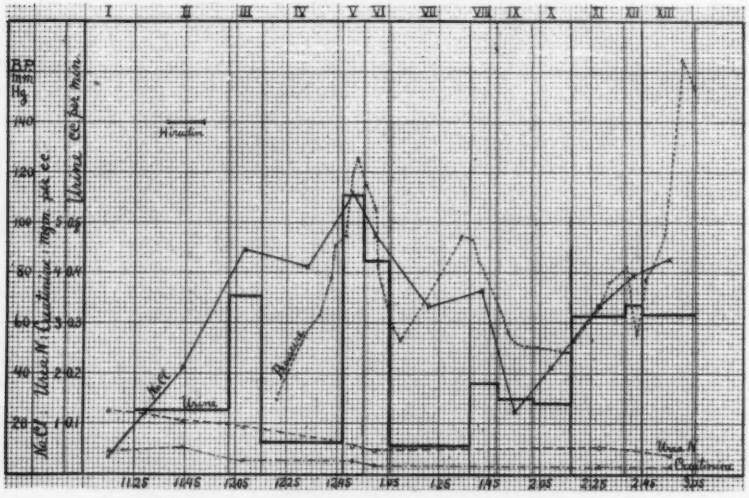


Chart 2. Perfusion pressure, urine flow, and concentration of chloride, urea, and creatinine in experiment I. Blood collected at the end of the experiment contained chloride in concentration equal to 5.98 mgm. NaCl per cc.; urea, 0.126 mgm. urea N per cc. Urine I was collected from the bladder before evisceration; urine II from the ureter after evisceration. During period IX, 20 cc. 0.9 per cent NaCl were injected; during perod X, 3.5 cc. 5 per cent NaCl. The urine of period V was the first to show trace of hemoglobin.

per gram. The results show the retention by the kidney of power to concentrate urea and creatinine.

Taken as a whole, the analyses give a basis for confidence that the method employed does not damage the kidney to such an extent as to render results obtained inapplicable to discussion of events in the normal kidney.

Experiment II. October 22, 1915. (Charts 3 and 4.) In this experiment perfusion was begun at 11:42. After the initial adjust-

ment of the thrust of the pump at 11:48 no change in delivery was intentionally produced until 1:30. Such variations as did occur were due to inconstancy of the electric current. The steady increase in perfusion pressure which occurred between 11:50 and 12:16 was due to spontaneous constriction of kidney vessels resulting possibly from faulty artificial respiration. The splanchnic nerves were not cut.

The abrupt rise in perfusion pressure which occurred at 12:16 was intentionally caused by drawing 9 cc. of blood from the animal's circulation. The animal's carotid pressure fell at once from 92 to 40 mm.; perfusion pressure rose at once from 95 to 110, and in less than one minute later to 150. The renal constriction was certainly caused either by central (ischemic) stimulation of vasoconstrictor nerve fibers to the kidney or to the secretory mechanism of the suprarenal glands. When the 9 cc. of blood were returned to the circulation at 12:34 a partial return of blood pressure and perfusion pressure to their former values occurred.

The later course of the experiment is complicated by the introduction of relatively large amounts of reserve blood which had been standing at room temperature for 2 hours. We took occasion to test the effect of introduction of sodium chloride solutions, first 4 cc. of 5 per cent, later 23 cc. of 0.9 per cent. Both caused diminution in perfusion pressure (vasodilatation) and increased diuresis. The results are illustrative of the fact that changes in composition of the blood may induce changes in urine formation irrespective of velocity of blood flow and height of renal blood pressure.

The outstanding fact of importance is that step by step with the increase and subsequent decrease in perfusion pressure, in the earlier part of the experiment, parallel changes in rate of urine output occurred, despite the fact that blood flow through the kidney did not change. It is, perhaps, unfortunate that we have not analyses of blood composition before and after the 9 cc. hemorrhage which would enable a statement concerning possible hydremia produced by the hemorrhage. Consideration of other experiments removes the possible force of this omission.

In this experiment, chlorides of the blood were equivalent to 0.58 per cent NaCl; blood urea N was 0.011 per cent. Bladder urine contained chloride equal to 0.57 per cent. Hence we may believe that this rabbit differed from that of experiment I in respect of chloride saturation of the tissues. At the beginning of that experiment, in relation to water chlorides were retained; at the beginning of this,

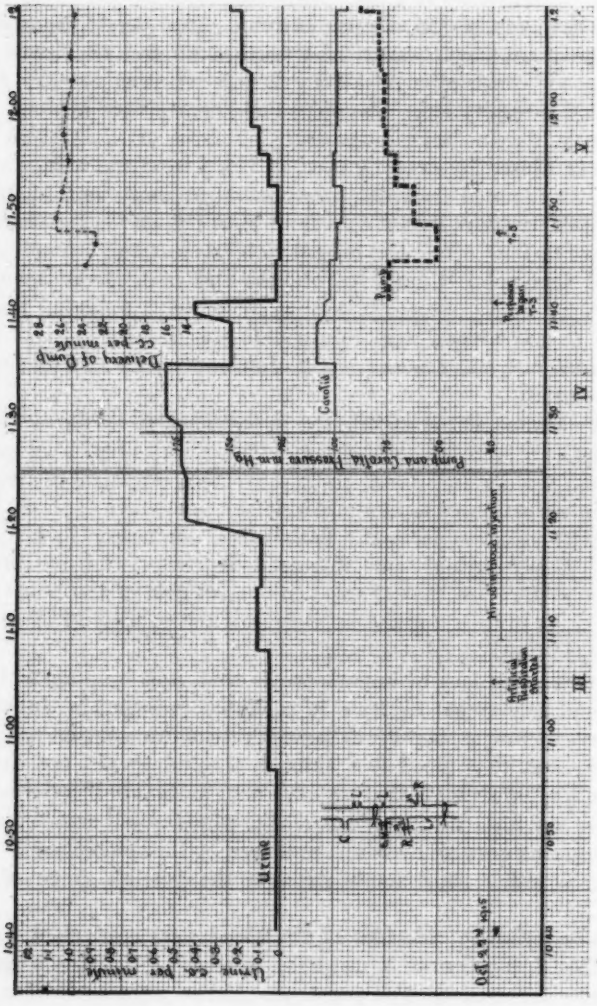


Chart 3 (left half)

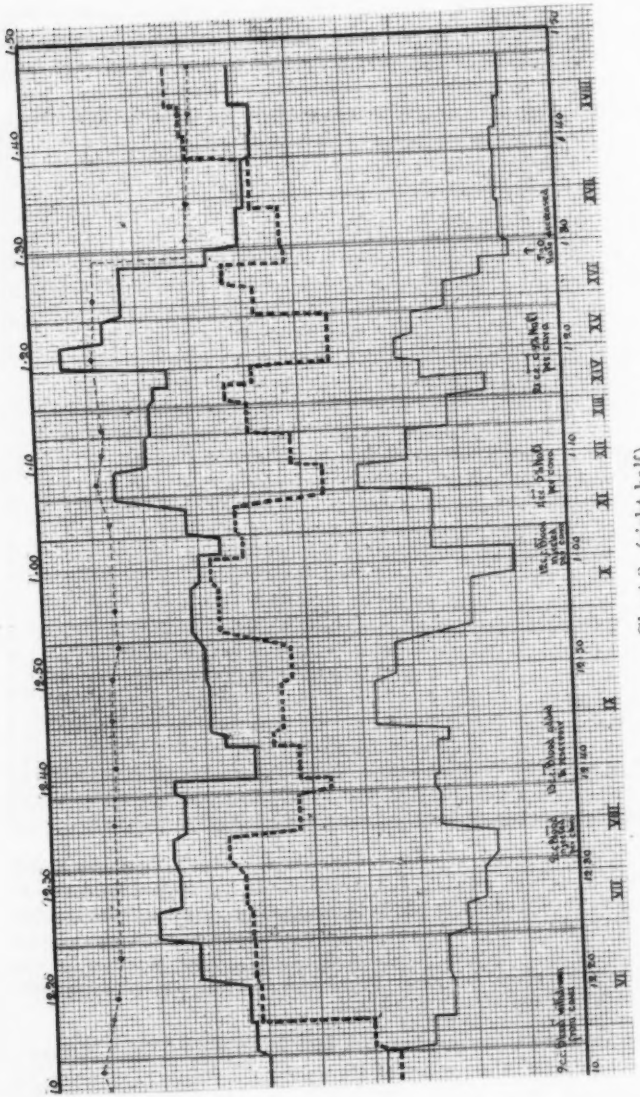


Chart 3 (right half)

Chart 3. Experiment II, October 22, 1915. Perfusion of kidney at constant rate of blood flow, varying pressure. Splanchnic nerve not cut.

chlorides were eliminated. During the first perfusion period (V), urinary chloride rose to 1.24 per cent, more than double that of the blood. It is possible that the action of hirudin (see page 176) had

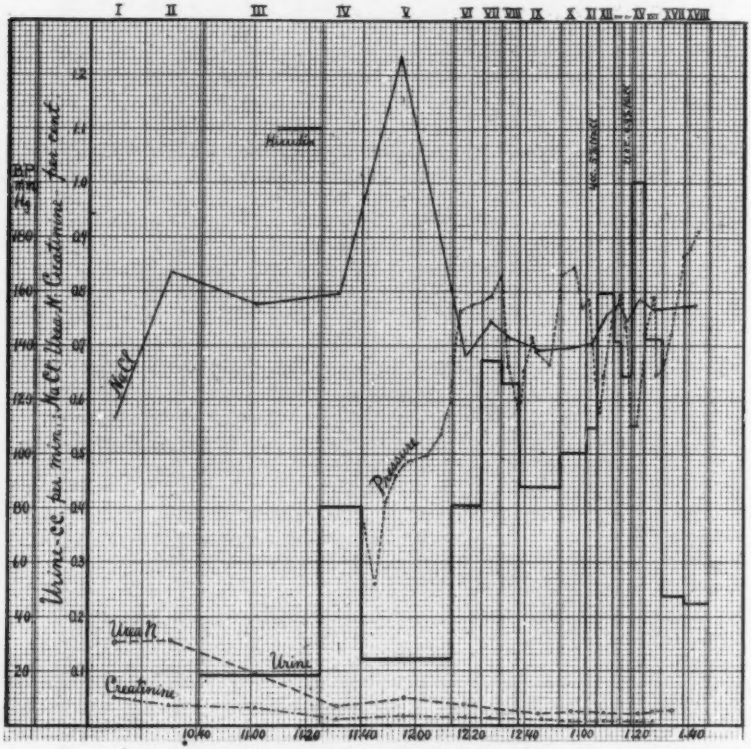


Chart 4. Perfusion pressure, urine flow, concentration of chlorides, urea N and creatinine in experiment II. Blood at end of experiment contained chlorides equivalent to 5.8 mgm. NaCl per cc; urea N = 0.113 mgm. per cc. Urine samples I and II were collected from the bladder before and after evisceration respectively. Urine III was collected largely before the injection of hirudin. Urine of period VI was first to show a trace of hemoglobin; that of period IX showed a few red blood cells. Urine of period XVIII contained much blood.

something to do with this rise, but the fact is apparent that the perfused kidney exhibited the power of eliminating a solution of chloride more concentrated than blood. In subsequent periods chloride fell to the region of its concentration in blood plasma.

Fluctuations in urea N and creatinine were similar to those in experiment I. Throughout the experiment both were eliminated in the urine in more concentrated solution than in the blood. We regard these two experiments as exhibiting the retention of power by the perfused kidney to adjust the excretion of water and chloride in relation to the condition of the body, and hence as evidence that no great perversion of function occurs when perfusion is established.

Experiment III. July 18, 1916. (Charts 5 and 6.) In the third experiment the results are rather more striking than in any other. In the first 9 minutes of perfusion the output of the pump was increased because urine elimination practically ceased at the beginning of perfusion. From that time on no change was made in the adjustment of the pump. At the outset of the perfusion the animal's corneal reflex was active, he made rather violent respiratory movements despite artificial respiration. These movements were quieted by giving ether via the respiration pump at 11:49. Associated with these changes in the animal's condition conspicuous changes in perfusion pressure and in urine flow occurred. Pressure increased from 85 to 170 mm. and urine from just above zero to 0.65 cc. per minute (fig. 2). Blood flow through the kidney was constant. The steplike increase in perfusion pressure preceded a parallel increase in urine. Neither can be regarded as a delayed effect of the initial increase in perfusion volume because of the subsidence of both effects following ether administration. Here again, as in experiment II, we are confronted with a spontaneous change in the caliber of renal vessels. Careful search at the end of the experiment showed that the splanchnic nerves on both sides had been successfully cut. We can conceive of no other explanation for this conspicuous constriction and subsequent dilatation of renal vessels than central stimulation of constrictor nerve fibers to the kidney which was abolished by increasing depth of anesthesia. It is commonly held that the majority of such fibers reach the kidney via the splanchnics. Cohnheim and Roy (17), however, showed that renal vasoconstriction resulting from asphyxia or sensory stimulation occurred when both splanchnic nerves were cut; and Jost (18) has published experiments indicating the existence of other vasoconstrictor paths to the kidney than the splanchnic nerves. Our experiment supports this evidence.

The injection of 4 cc. of fresh blood into the animal's circulation at 12:01 hastened the production of relaxation of kidney vessels. There is no reason to suspect in this very fortunate result that an alteration in blood composition occurred. If the correspondence of urine and

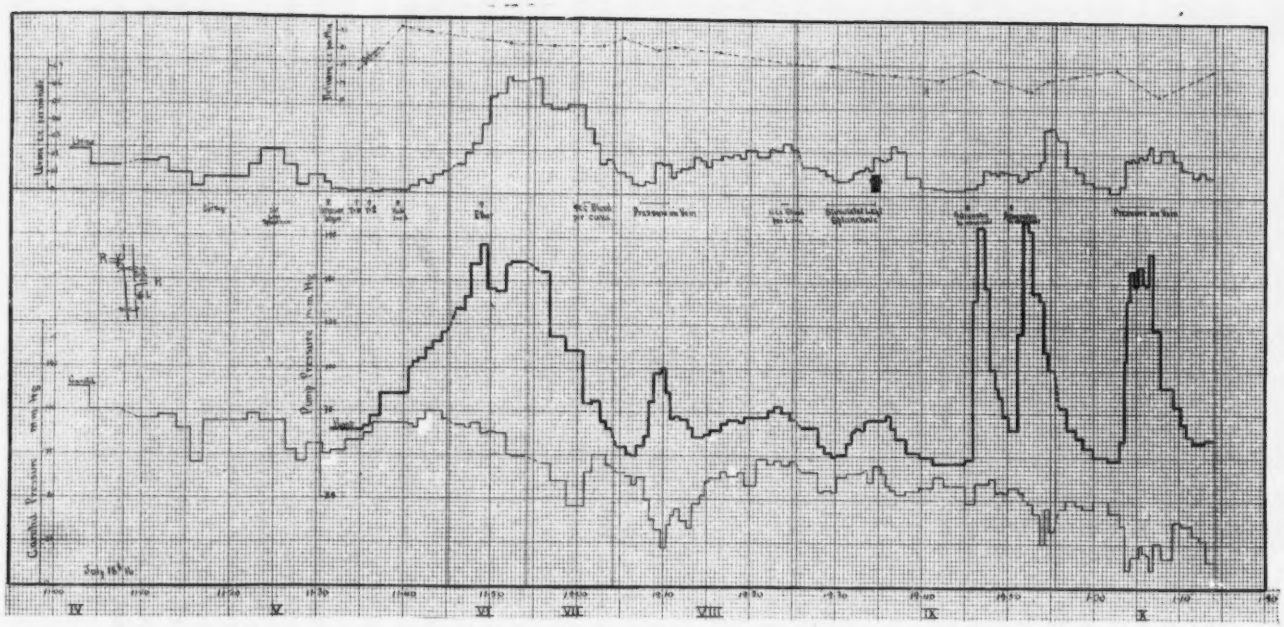


Chart 5. Experiment III, July 18, 1916. Perfusion of kidney at constant rate of blood flow and varying pressure; action of adrenalin and of pressure on the renal vein. Splanchnic nerves cut.

pressure curves in this experiment in which blood flow was constant be compared with that in chart 1, in which both flow and pressure were increased, the conclusion is unescapable that the variations in blood pressure have more of a determining influence under these conditions of experimentation than do variations in blood flow.

Later in the experiment, at 12:07, pressure in the renal vessels was increased by laying a finger lightly on the renal vein so that perfusion pressure (renal artery) rose from 52 to 100 mgm. Hg. At 12:29 the

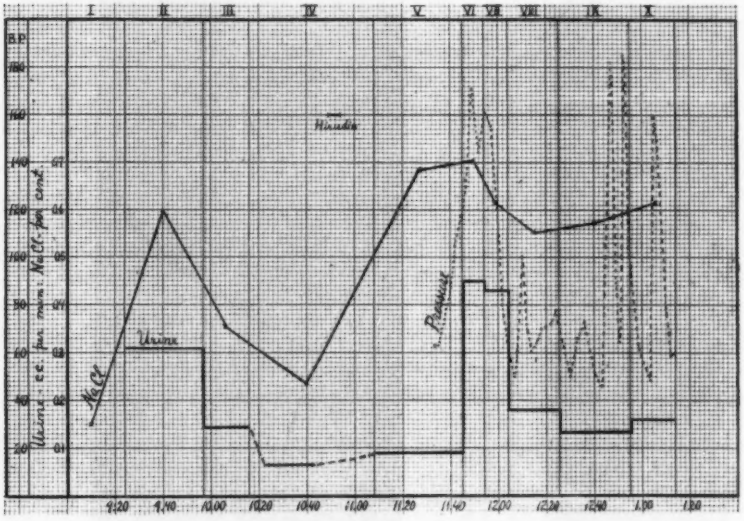


Chart 6. Perfusion pressure, urine flow and urinary chloride concentration in experiment III. Urines I, II and III were collected from the bladder, I before, II and III after evisceration. Urine of period VII was first to show hemoglobin; that of VIII showed red blood cells; urine of period X contained considerable blood.

peripheral stump of the left splanchnic was stimulated electrically. Perfusion pressure rose from 50 to 74. At 12:46 and again at 12:51 adrenalin was added to the intake reservoir of the pump and perfusion pressure rose from about 50 to more than 175 mm. At 1:04 and finally at 1:14 (fig. 3) the experiment of compressing the renal vein was repeated. Urine flow increased in response to each of these changes. Variations in blood flow through the kidney were inconsiderable; the greatest occurred toward the end of the experiment. There is no in-

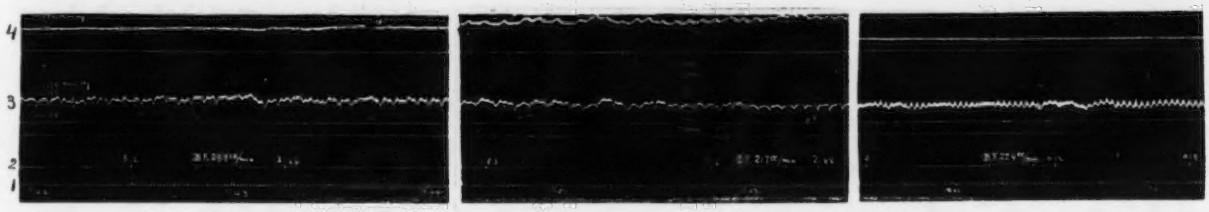


Fig. 2. Portions of the original record of experiment III showing effects of the spontaneous constriction and subsequent relaxation of renal vessels upon perfusion pressure and urine flow. *1*, Time in seconds; *2*, signal showing urine readings in graduated cannula connected with ureter and blood flow estimations (time required for the pump to deliver 5 cc. of blood); *3*, carotid blood pressure; *4*, perfusion pressure.

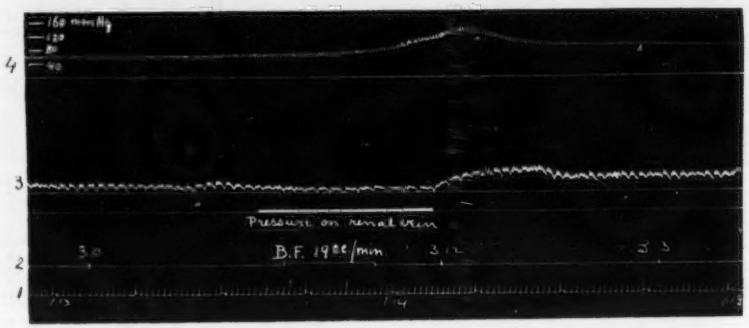


Fig. 3. Original record of experiment III showing effect of slight compression of renal vein. Curves are numbered as in figure 2.

dication that they are concerned in the alterations of urine, for a slight increase occurred during the first adrenalin test, and a decrease during the final test of compression of renal vein.

The impression which the whole record gives is that the curve of renal blood pressure is faithfully reflected in the curve of urine elimination.

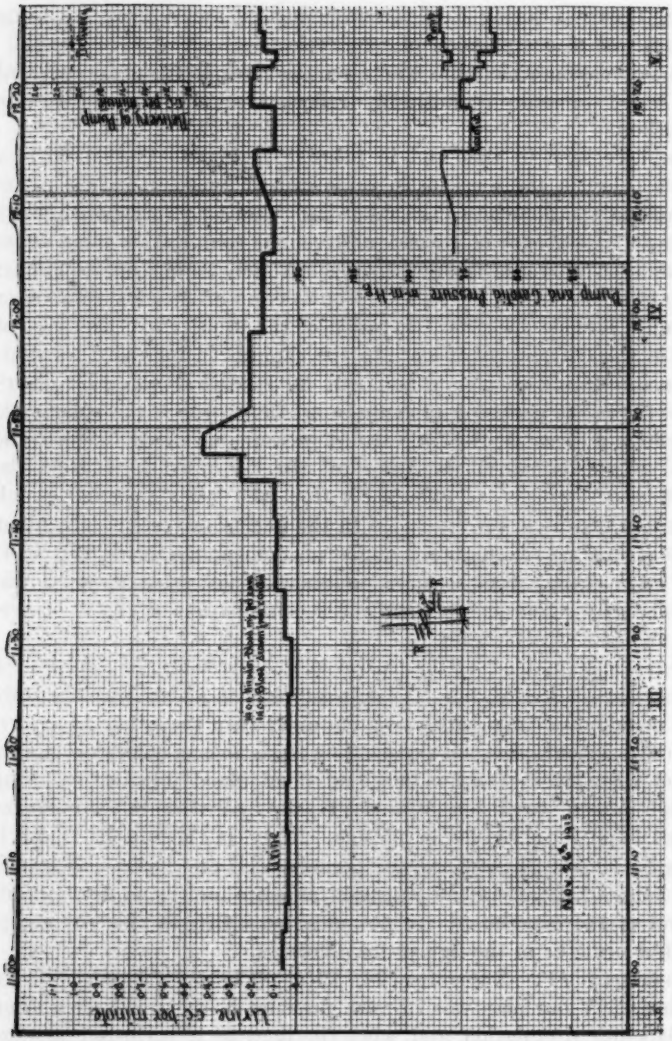
It will be seen in this and in other experiments that there is a sensible delay in the increase in urine caused by splanchnic stimulation and adrenalin while that following venous compression is very prompt. Turgescence of vessels from venous compression must cause narrowing of lumina of tubules; this causes increase of urine to appear promptly in the ureteral cannula. Constriction of vessels on the other hand may provide space for expansion of the tubules with consequent delay in the entrance of urine into the cannula in increased amount.

It is interesting to note that about 30 seconds before the beginning of the rise in perfusion pressure from stimulation of the splanchnic at 12:30 a noticeable rise in carotid pressure occurred. Since the animal was eviscerated the cause of the rise must have been the liberation or secretion of epinephrin from the left suprarenal gland, the vessels of which were included in the perfusion system. In other tests of splanchnic stimulation a similar phenomenon occurred (see fig. 4) hence we must regard the constriction following nerve stimulation in this and following experiments as really the combination of effects of nervous and chemical stimuli.

Attention should be called to the sketch showing the position of the ligature on the aorta. The left suprarenal artery was the only vessel included in the perfusion system other than the left renal artery.

Urine analyses in this experiment show variations in chlorides roughly parallel with those of urine flow and renal blood pressure. During the high pressure perfusion (VI and VII) the figures are in the region of concentration of chlorides in plasma. They fell definitely below it during the subsequent low pressure periods (VIII and IX). The transitory changes induced in the perfusion pressure and urine flow toward the end of the experiment are not reflected either in this condensed record of urine or in the figures for chlorides.

Experiment IV. November 26, 1915. (Charts 7 and 8.) In experiment IV our plan was to raise pressure in the renal circulation by splanchnic stimulation, and, when the increase in perfusion pressure had been attained, to maintain it after cessation of splanchnic stimulus by increase in output of the pump. Such a plan, if successfully


Pump line Gauge Pressure in lbs.
Gauge
Nos. 2 & 3 Wells
Nos. 1 & 4 Wells

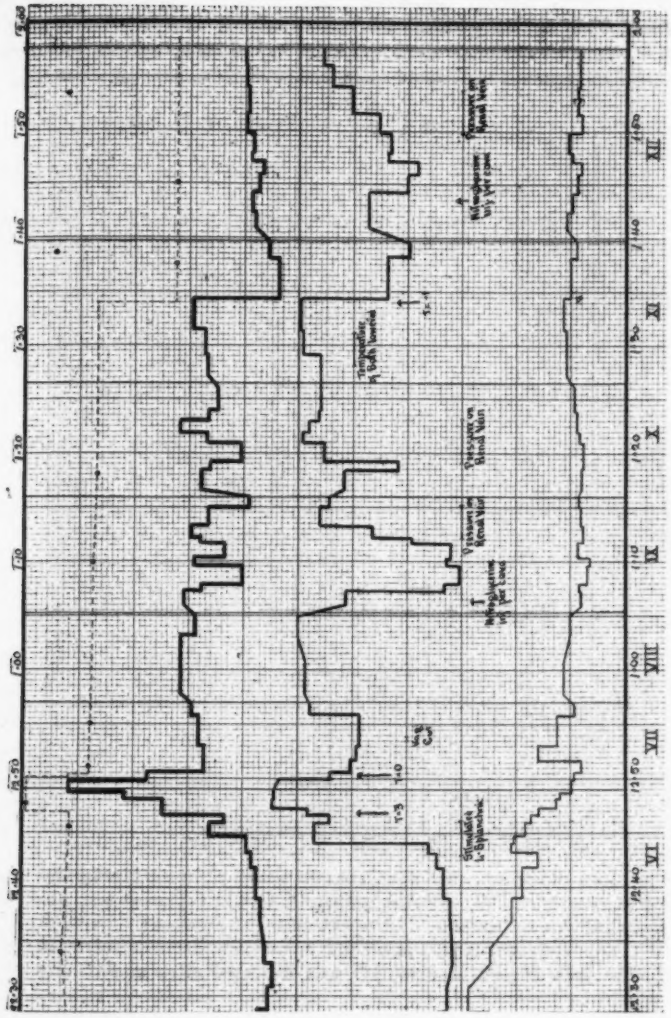


Chart 7 (right half)

Chart 7. Experiment IV, November 26, 1915. Perfusion of kidney. Effect of splanchnic stimulation, blood flow constant. Attempt to compare effect of change of pressure with that of change of flow. Action of nitroglycerine. Spanchnic nerves incompletely cut. (The notations "$T=3$", "$T=0$", etc., on this and other charts refer to adjustments of the thrust of the pump, expressed as divisions of an arbitrary scale etched on the driving arm of the pump mechanism.)

accomplished, would indicate the relative effectiveness of increase of blood flow as compared with increase of blood pressure in increasing urine. The effort was unsuccessful by reason of the fact that we had not sufficiently recognized and hence did not wait for the lag in in-

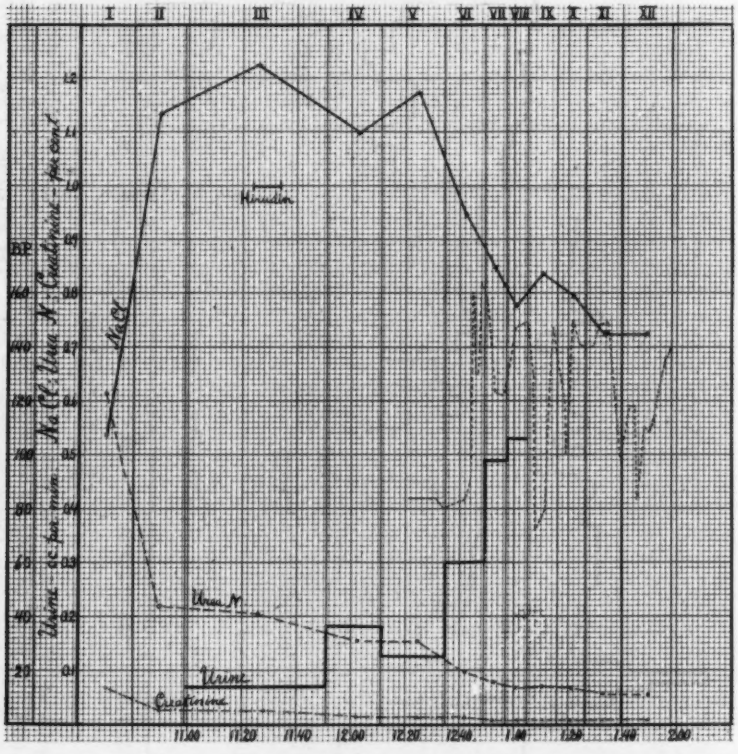


Chart 8. Perfusion pressure, urine flow, concentration of urinary chlorides and creatinine in experiment IV. During periods I, II and III urine was collected from both kidneys and combined; flow calculated as from one. Urine of periods I and II was collected from the bladder before and after evisceration respectively. The greater part of urine III was collected after injection of hirudin.

crease of urine which follows rise of pressure caused by splanchnic stimulation. For this cause we over-compensated and are unable to say whether the further increase in urine which occurred after 12:47 was due to increase of pressure or of flow.

The record is worth presenting, however, because of the unmistakable increase in urine which followed uncomplicated rise of pressure from splanchnic stimulation between 12:43 and 12:47, and also because it shows an effect from nitroglycerine. The two trials of compression of the renal vein gave results similar to those pointed out in experiment III, but are not conspicuous enough to warrant especial emphasis.

The urine analyses gave results analogous to those of experiment II. Chloride content of urine collected from the bladder was high (0.53 per cent); evisceration and hirudin increased it to 1.22 per cent. It remained high during the first two perfusion periods (V, 1.17; VI, 0.95) and then sank to the region of chloride concentration in rabbit's blood plasma. The fluctuations which occurred during periods VII, IX and X are such as would be expected if the kidney were retaining water in preference to chlorides; the high chloride figures at the beginning of the experiment indicate that this was the case.

Urea N was determined in these samples by the Folin method; in previous experiments the Van Slyke method was used. The high figures here are due to the fact that in Folin's method urethane reacts like urea, hence these figures include the nitrogen of both substances. The fluctuations of urea N and creatinine in this experiment are like those previously observed.

Experiment V. June 6, 1916. (Chart 9.) Splanchnic stimulation was tested twice and pressure on the renal vein once, all with positive results.

Experiment VI. July 11, 1915. (Chart 10.) Two ligatures were placed about the aorta; one just below the coeliac axis, the other between the two renal arteries. Perfusion was begun with the upper ligature tied; at 11:58 the lower ligature was tied. This shut off from the perfusion system two rather large lumbar arteries and consequently increased flow and pressure in the kidney. This change was compensated by diminishing the thrust of the pump so that output was lessened from 24 to 15.5 cc. per minute. Splanchnic stimulation after this change in the perfusion was not as effective as before, either because constrictor fibers to the kidney were included in the second ligature or because the condition of the nerve where the electrodes were applied had deteriorated.

Compression of the vein was effective at 12:29 to 12:35.

Urine samples were analyzed for chlorides and creatinine; the results add nothing to those already cited and hence are omitted.

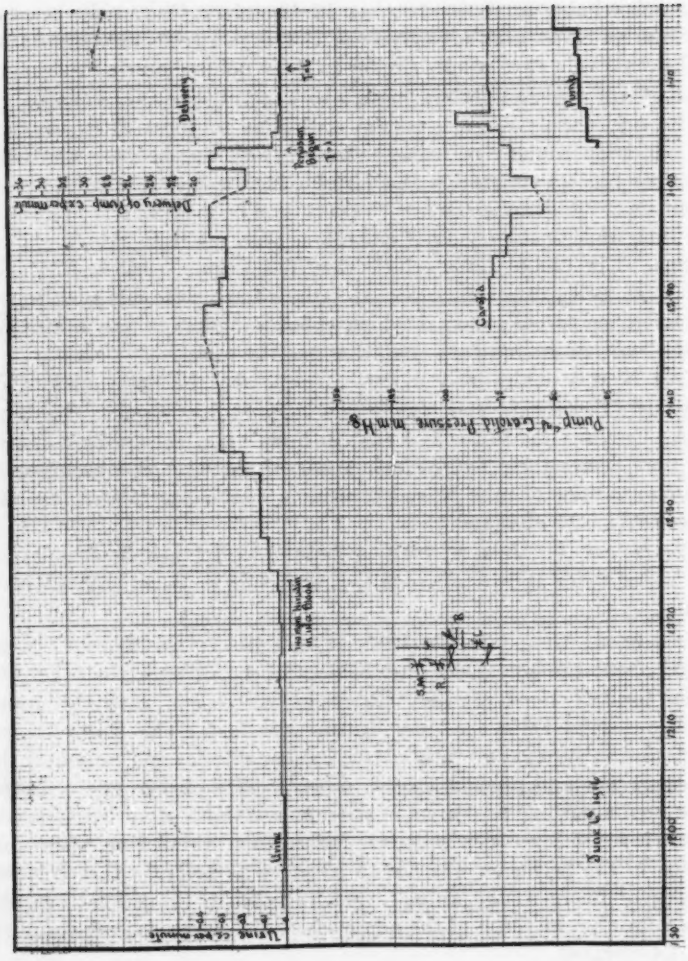


Chart 9 (left half)

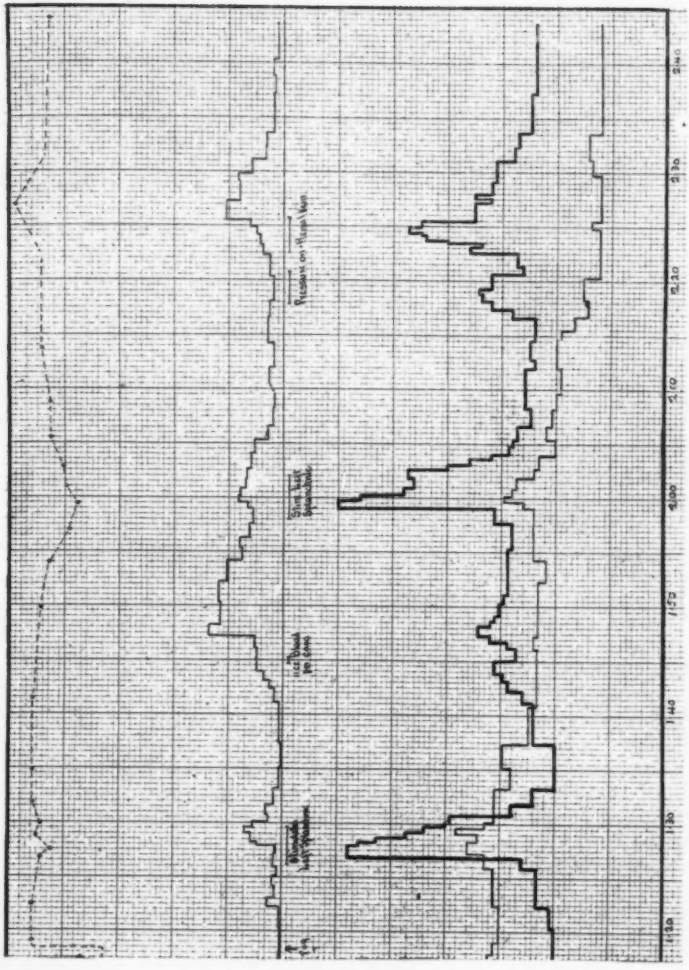


Chart 9 (right half)

Chart 9. Experiment V, June 6, 1916. Perfusion of kidney, blood flow constant. Splanchnic nerves cut. Effect of stimulation of splanchnic and of compression of renal vein.

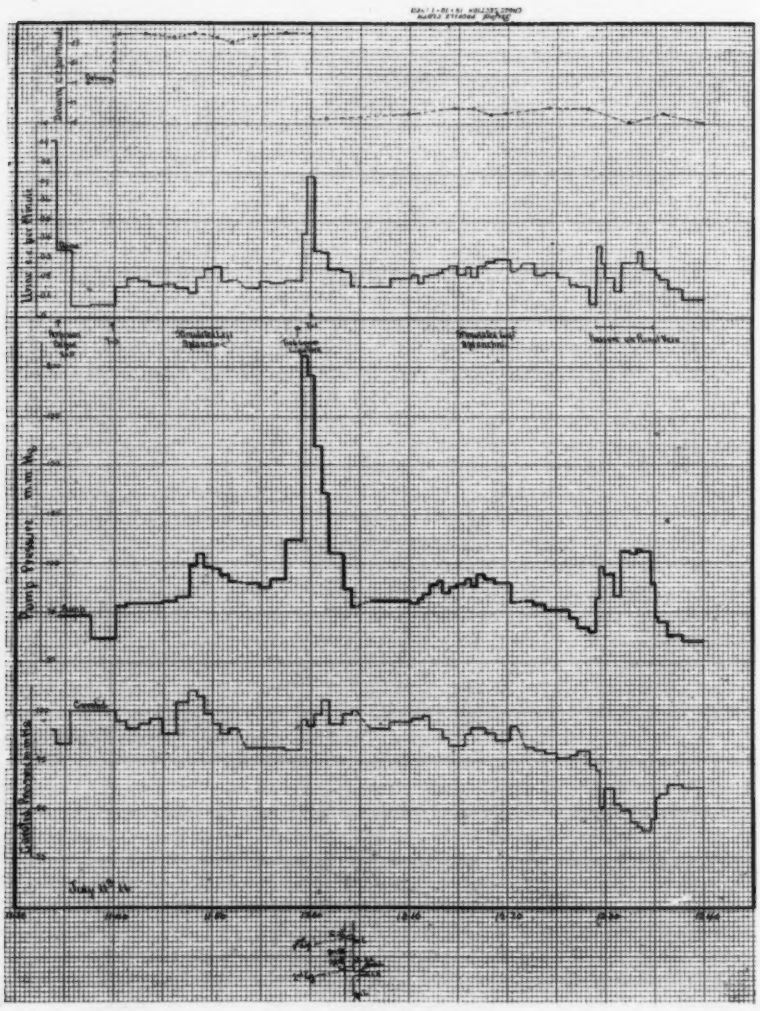


Chart 10. Experiment VI, July 11, 1916. Perfusion of kidney at constant rate of blood flow. Splanchnic nerves cut. Stimulation of splanchnic. Pressure on renal vein.

Experiment VII, July 15, 1916. (Chart 11, figs. 4 and 5.) This shows in striking fashion the results of stimulation of the splanchnic and of the injection of adrenalin. It is especially interesting to note that in

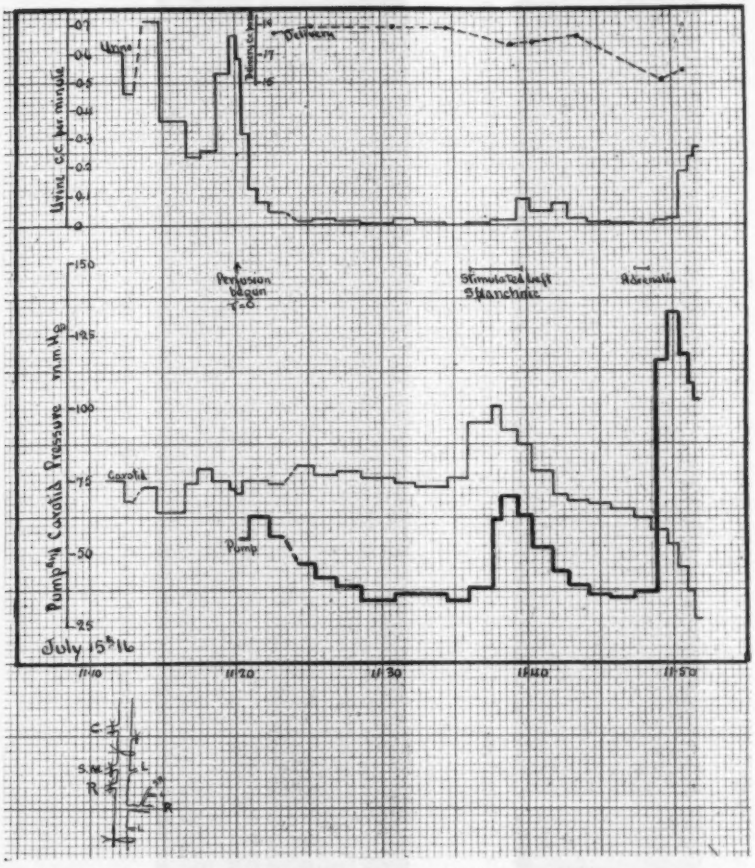


Chart 11. Experiment VII, July 15, 1916. Perfusion of kidney at constant rate of blood flow. Splanchnic nerves cut. Effects of splanchnic stimulation and of adrenalin.

this experiment the output of the pump was relatively low so that pressure in the renal circulation was just about at the threshold of that necessary for urine elimination (35 to 37 mm. Hg. from 11:27 to 11:37).

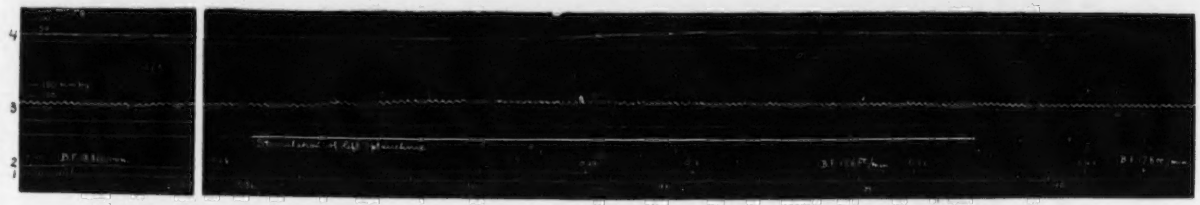


Fig. 4. Original record of effects of splanchnic stimulation in experiment VII. Curves numbered as in figure 2.

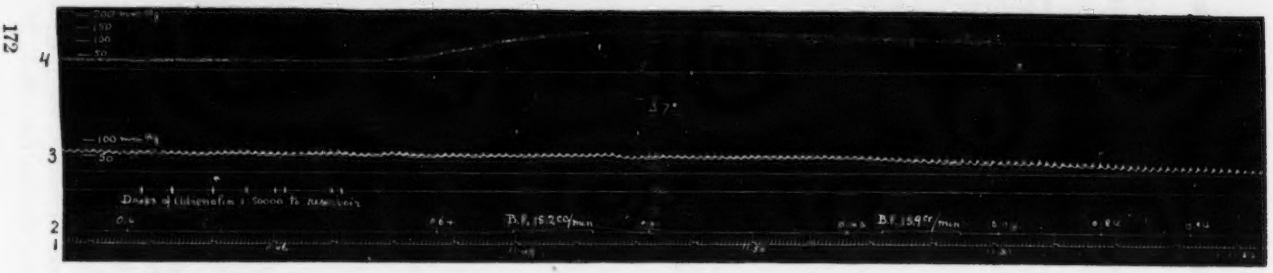


Fig. 5. Original record of effects of introducing adrenalin into renal circulation in experiment VII. Curves numbered as in figure 2.

This figure corresponds well with the figures for minimal blood pressure at which urine is eliminated as determined by others (19), and with Starling's figures for osmotic pressure of blood proteins (20). The fact that striking increase in urine was secured by the two agencies used to increase pressure without significant change in blood flow is important in that it permits the belief that increase in pressure is not only capable of increasing rate of urine flow already established but is also capable of causing urine to flow from a kidney which had previously been eliminating nothing; in other words, this experiment has a somewhat more direct bearing on renal blood pressure as a *cause* of urine elimination than the others.

CRITICAL CONSIDERATIONS

Before stating the conclusions which these results appear to warrant it is proper that attention should be directed toward such defects as are inherent in the plan of experimentation or were introduced through error.

1. While we think that the perfused kidney has preserved its normal powers to a satisfactory degree, it would be idle to claim that we have been dealing with a normal kidney. By evisceration the normal relationship between renal circulation and that in the rest of the body is upset. A plethora of the remaining vessels is induced; this includes the renal vessels. This plethora produces diuresis[3] and with it comes an impressive increase in urinary concentration of chlorides. Both diuresis and excess chloride elimination commonly subside together. In some experiments undertaken to throw light on this phenomenon alone (charts 12 and 13), the curve of chloride concentration is so strikingly parallel to that of urine flow as to indicate their strictest interdependence. In others (charts 14 and 15), with slow urine flow, there is no relationship between the two. In some of the experi-

[3] The results of Magnus (Arch. f. exper. Path. u. Pharm., 1901, xlv, 210) are commonly cited as evidence that plethora alone does not cause diuresis. He induced plethora by transfusion from one animal to another, both having been kept under identical dietary conditions for some days before the experiment. In our experiments it seems that a plethora is just as truly produced by ligation of the great arteries of the gastro-intestinal tract as in his by transfusion of foreign blood: diuretic response to this ligation is practically instantaneous, making it difficult to believe that an alteration in composition of the blood could be responsible. It will be noted that Magnus used animals which had been deprived of water before the experiment while ours were diuretic to begin with; and that in ours, no foreign blood was introduced at this stage of the experiment.

ments in which evisceration alone was studied, red blood cells appeared in the urine as a consequence of it. While such evidence as we have indicates that the disturbance caused by evisceration is transitory and does not commonly include especial damage to the kidney, the possibility exists that removal of the gastro-intestinal tract may take away

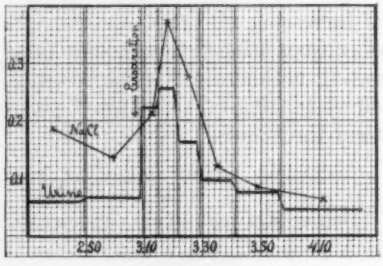


Chart 13

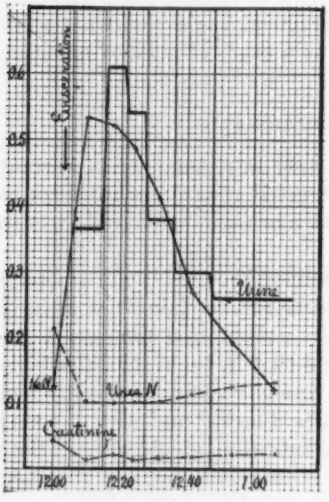


Chart 14

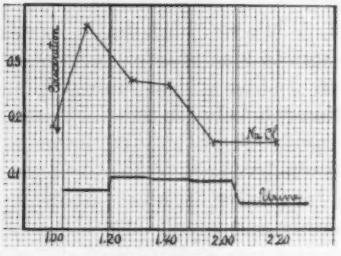


Chart 15

Chart 12. Experiment of September 7, 1917. Effect of evisceration upon urine flow, and urinary concentration of chloride, urea and creatinine. Ordinates represent cubic centimeters of urine per minute and percentage of chloride (calculated at NaCl), urea N and creatinine. Abscissa shows time. The first values charted at 12:00 o'clock, represent urine taken from the bladder at beginning of operation. No urine was eliminated from then until ligation of coeliac axis, superior and inferior mesenteric arteries and right renal artery at 12:01.

Chart 13. Experiment of May 29, 1917. Effect of evisceration upon urine flow, and chloride concentration. Abscissa shows time, ordinates urine flow per minute and chloride concentration in per cent. This rabbit received 50 cc. 6 per cent of glucose solution by stomach tube 3 hours before the experiment. All urine samples here shown were collected from the ureter. Concentration of chlorides in bladder urine was the same as that of first sample charted.

Chart 14. Experiment of October 19, 1917. Effect of evisceration on urine flow and chloride concentration. The first value of chloride concentration shown is for urine from the bladder, rate of elimination of which was not known.

a source of some substance which takes part in the normal adjustment of renal elimination to bodily conditions. We must concede that an eviscerated animal is not a normal animal.

2. It is apparent also that the use of hirudin introduces another unknown factor. We used the preparation made by Sachse & Company and this has been shown by Marshall (21) to possess very definite toxicity for dogs; much less for rabbits. That it is not productive of

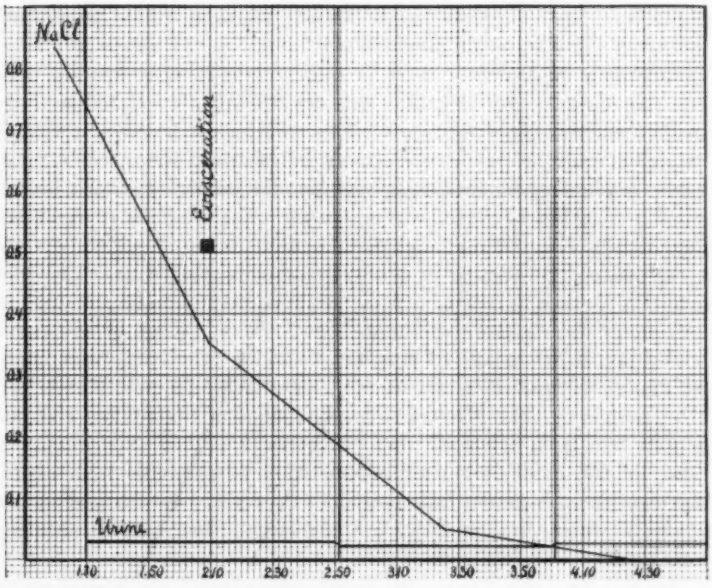


Chart 15. Experiment of May 4, 1917. Effect of evisceration on urine flow and chloride concentration. Abscissa shows time; ordinates represent urine flow in cubic centimeters per minute and percentage of chloride calculated as NaCl. First chloride value charted is from bladder urine.

gross alteration in the circulation of our rabbits is shown by the figures for carotid blood pressures in the several experiments at the beginning of perfusion; they are well within the range of those encountered in animals similarly prepared but which received no hirudin. One experiment was made as a control on several of the features which our perfusion experiments included: evisceration, hirudin, drawing and reinjecting blood, and finally, during a period of 2 hours, bleeding

from the carotid and simultaneously reinjecting into the jugular at rates comparable to those at which the pump, in perfusion experiments, removed blood from and returned it to the animal's vessels. The blood pressure at the end of the experiment 4 hours after the injection of hirudin, was 88 mm. and at no time was there indication of failure of the circulation except when rate of bleeding was too great.

Sievert (22) states that commercial hirudin in rabbits causes albuminuria. His paper does not show whether he differentiated between hirudin itself and albumin in the urine. Hirudin added to normal urine gives the commoner albumin reactions.

In our own experiments there is some evidence that hirudin does affect the kidney. Its injection was practically always followed by diuresis even though it was injected in solution in blood and though an equal amount of blood was removed from the animal receiving it. In some of the experiments, after hirudin, the course of chloride elimination in relation to water was not quite that which was expected. In experiment III, the rise in chloride concentration in period V without corresponding increase in urine; in experiments VI and VII (not charted) a marked increase in urine with no corresponding change in chlorides; in the control experiment cited above, failure of the curve of chloride concentration to sink as it did in practically all control experiments without hirudin; these are instances in point, and lead to the suspicion that the renal mechanism for controlling chloride retention has been affected. On the other hand, in experiments I, II and IV the chloride concentration curve corresponds with expectation and in experiment III, periods VI to IX, the changes in the relation of water and chlorides are similar to those which occurred before hirudin was injected.

3. In exposing the defects of these experiments it is necessary to mention artificial respiration. We used a Meyer pump (23) (made by Rinck of Marburg) in the belief that it was a very perfect machine for the purpose. It consists of two cylinders, one for inflation, the other for deflation of the lungs. We discovered long after this work was done that the two cylinders are not of exactly the same size; hence, as used by us, a gradually progressive deflation of the lungs took place, which would give a gradually developing increase in resistance in the pulmonary circulation (24). We think that this was partly responsible for the progressive fall in the animal's pressure which occurred in the longer experiments.

4. In all of the experiments, hemoglobin appeared in the urine sooner or later. It was always preceded by the appearance of hemoglobin in the plasma and results, beyond doubt, from rupture of red cells in passage through the pump. While it is no indication of renal abnormality it does show damage to blood cells.

5. In most of the experiments the urines collected toward the end deposited on standing a slight sediment of red corpuscles. This fact does not necessarily show such a degree of damage as to materially lessen the force of these experiments. As soon as each experiment was concluded the kidney was sectioned; macroscopic examination never revealed anything grossly abnormal. Sections of fixed tissues were made for us by the late Dr. Alfred Allen and the reports of microscopic examination were "normal." This indicates that the minute hemorrhage represented by the red cells in the urine was glomerular and we believe was of the same character as that which is sometimes produced by evisceration alone. Considerations concerning the development of this condition late in an experiment are presented later.

6. Over against these defects we set the following facts: (*a*) No interruption in blood flow through the kidney occurred when the artificial circulation was substituted for the normal and in at least two of the experiments no interruption of urine elimination occurred. (*b*) In early periods of perfusion albumin reactions in urine were no more pronounced than during collections made before perfusion but after hirudin. Sugar was never detectable in any urine when tested by Benedict's reagent. (*c*) Figures for concentrations of urea and creatinine in urine compared with blood showed that the kidney during perfusion retained its power of eliminating these substances in higher concentration than that in which they exist in blood. Figures for chloride concentrations show variations both above and below blood concentration, a fact which may be interpreted as indicating retention of power on the part of the kidney to eliminate chloride in correspondence with the state of the body.

DISCUSSION AND CONCLUSIONS

Both the purpose of these experiments and the principle of the method used are extremely simple. If the normal functions have been sufficiently preserved in our perfused kidneys the results are clearly applicable to the problem of the nature of urine formation. Reasons for confidence in such preservation of normal function have been stated.

In one experiment, by changing the supply of blood to the perfused kidney and thus changing both velocity of blood flow and blood pressure in the renal vessels, changes in volume and composition of urine occurred similar to those which would have been expected in the urine from an intact animal whose kidney had been subjected to similar circulatory changes.

In six experiments, velocity of blood flow through the kidney was kept constant and pressure varied by a variety of means—medullary stimulation (ischemia); stimulation of the splanchnic nerve; introduction of adrenalin and nitroglycerine into the renal circulation; compression of the renal vein—and in the great majority of tests changes in urine volume were parallel with changes in renal blood pressure. This appears to be direct evidence that pressure, *per se*, is a cause of urine formation and so constitutes strong support of the filtration theory.

Two questions of fundamental importance must be settled before correctness of this statement can be granted: First, the question of a possible path for the blood stream within the kidney which does not pass through glomerular capillaries. Are there arterial branches within the rabbit's kidney which supply the tubules directly without interposition of glomeruli? If so, the implication of constancy of glomerular blood flow which we have adopted is not justifiable. We rely upon the work of Huber (25) on this question, supported by the subsequent observations of Gerard (26) and of Gross (27). In it he was unable to find evidence of existence of *arteriolae rectae verae* in the rabbit's kidney.

The second question is the outcome of later work in this laboratory by Schmidt and one of us (28) in which the glomerular circulation in the frog's kidney has been subjected to direct observation. It may be stated in this way: Is the number of glomeruli, which receive blood throughout the changing phases of our experiments, constant or varying? If varying, then the constancy of flow through the whole kidney is deceptive. Driving a constant amount of blood per minute through a varying number of glomeruli would certainly result in varying rates of blood flow through individual kidney units.

In the work alluded to it has been found that under conditions of moderate blood flow and scanty diuresis only a fraction of the glomeruli receive blood. This number may be decreased by injection of constrictor doses of adrenalin. It has been found also that when the frog's kidney is exposed and damaged as by the tedious dissection necessary for preparing its blood vessels for perfusion and the ureter for collection of urine, inspection may reveal no glomerulus showing

active circulation. If, under these conditions, a perfusion stream of hirudinized frog's blood be forced through the vessels it flows for a few moments at moderate pressure, then the perfusion pressure rises inordinately and blood begins to ooze from the entire surface of the kidney. The preliminary treatment, which includes exposure, reduced circulation and hirudin, has caused closure of such a large fraction of the renal vessels that adequate perfusion is impossible.

If the results of these experiments with the rabbit's kidney are examined with this information in mind, analogous phenomena appear.

In experiments I, II and III, pressure tends to rise markedly toward the end of the experiment. In our collection of records of unsuccessful experiments, not included in this report, a striking feature has been the rapid increase of perfusion pressure to such a height as to make continuance of the experiment impossible. We came to feel that the chance of a successful result was dependent upon the rapidity and dexterity with which preliminary operations were done, and then that results to be reliable should be obtained within the first 30 to 40 minutes of the perfusion. For this reason the order of procedure in different experiments differed, and for this reason little has been said in this paper about events which occurred late in a long experiment.

During the course of such an experiment as those which we have described there may be a progressive development of influences which will close the circulation in a greater and greater number of units of the kidney (renal vasoconstriction from low blood pressure in the animal; adrenalin; substances released from damaged corpuscles; hirudin (?)). In consequence all of the blood which at first was flowing through many glomeruli must now flow through a few (relatively). Hence, not only glomerular pressure but also glomerular blood flow must rise. The volume of filtrate from each glomerulus must increase in inverse ratio to the number functioning, and with increase in rate of glomerular filtration goes decrease in time available for elaboration of the filtrate in the tubules either by absorption or secretion. For these reasons the fluid issuing from the ureter comes to take on more and more the character of a blood filtrate. The analytical figures for urines collected toward the end of our experiments bear out this view; and the appearance of red blood cells in the urine is a sign that some glomerular capillaries are being subjected to greater pressure and distention than they can bear without injury.

If these considerations apply to later events in our longer experiments, it might be objected that they are applicable to those changes

in perfusion pressure and urine flow upon which we are laying the most emphasis. It may be that in the intact animal renal vasoconstriction produced by medullary stimulation, splanchnic stimulation or by adrenalin is always bound up with a restriction in number of glomeruli which receive blood; if this were true in these perfusion experiments, then obviously the problem which we set for ourselves has not been solved. We made the experiments with the idea which is tacitly current that the circulation in the kidney is at least approximately uniform throughout its units, and that a constrictor influence on the kidney is uniformly distributed throughout them. The conception that the number of active glomeruli is variable introduces a new element into our discussion. If all of the glomeruli of the kidney received blood at one time, and if a constrictor influence affected the vessels all alike, than the conclusion would be unescapable that in these experiments pressure variations, apart from variations in velocity of flow, determine urine formation. But since we must take account of possible changes in the extent of blood channels it is necessary to inquire whether this change destroys the validity of our evidence. In the intact animal we feel sure that such a constriction as is represented by the more conspicuous of our tests of vasoconstriction in the perfused kidney (splanchnic stimulation and adrenalin) would be associated with diminution in number of glomeruli receiving blood. But these experiments in which the motive power of the kidney's circulation is a mechanical pump, indifferent to the resistance opposed to it, it seems certain that this diminution could not occur to such an extent. In the experiment of July 18, particularly, it is evident that even slight changes in renal pressure are reflected by parallel changes in urine. If the issue between blood pressure and blood flow as factors in urine formation were as clear-cut as is indicated in the Ludwig-Heidenhain discussion, and if velocity of flow (in the Heidenhain sense) and not pressure were the predominating factor, it appears that the strict parallelism just alluded to could not have occurred. Under the conditions of our experiments increased pressure must have been associated with distention and widening of glomerular capillaries, and hence with actual diminution in velocity of flow. For this reason, if velocity of flow in glomerular capillaries were the more important, then slight increase in blood pressure in these experiments should produce slight decrease in urine. Since in the great majority of instances it did not, we regard the evidence as competent to prove the essential importance of pressure. Furthermore, it would be extraordinarily difficult to

explain the increase of urine following increased venous pressure on the supposition that velocity of flow exerted as great a degree of influence on urine formation in these experiments as did renal blood pressure.

The question of decrease or increase in number of glomeruli receiving blood in the perfused kidney under the influence of constrictor or dilator influences is one which we believe to be answerable by direct experiment. Until such experiments shall give reason for the contrary we shall hold the view that in the work described in this paper approximate constancy of blood flow through the renal units was attained and hence that the changes in urine which were associated with changes in renal blood pressure were caused by them.

In the older work in the Ludwig laboratory changes in arterial pressure produced changes in renal blood flow and renal blood pressure simultaneously; in the original discussions arguments were presented for the view that the associated urinary variations were due to the changes in pressure (see page 145). These arguments receive strong support from all of our results in which the perfusion pressure was altered by agencies which act on the arterial muscle, i.e., medullary stimulation, splanchnic stimulation, adrenalin, nitroglycerine (see following paper). On the other hand our results with compression of the renal vein show that Heidenhain's denial of glomerular filtration because compression of the renal vein diminishes urine was not justified. For when conditions are established, as in our experiments, such that the obstacles to filtration which venous obstruction in the intact animal entails can not operate (compression of tubules, valve-like action of glomerular vessels, stagnation of blood in glomerular capillaries) increase of venous pressure increases urine.

In stating the conclusion which issues directly from these experiments, that renal blood pressure, dissociated from velocity of renal blood flow, exercises controlling influence upon the amount of urine eliminated and hence is to be regarded as a "cause" of urine formation, we are reversing an opinion previously expressed as the result of our experiments with caffeine. It may be said at once that the experiments here reported were technically far more perfect than those. By referring to the charts it may be noted that in four of our seven experiments, no additional blood was introduced into the perfusion system or into the animal's vessels after the beginning of the experiment. In others it was not needed before 40 to 60 minutes of perfusion had elapsed. This circumstance may be regarded as evidence of greater perfection of technique. In two of

the three caffeine experiments it was necessary to add new blood early in the experiment in order to maintain a circulation in the animal sufficient to yield blood to the pump. Study of those records indicates the disturbing influence of these additions and we believe that they masked the effects of changing pressure which might otherwise have been apparent. While we have little fear that repetition of the caffeine experiments will materially affect the chief conclusion of that paper concerning the action of caffeine, we feel sure that our provisional conclusion expressed concerning the relative importance of pressure and flow should be discarded.

It may be thought that the pains taken in these experiments to establish conditions, necessarily highly artificial, in order to differentiate between the importance of blood flow and of blood pressure in the kidney were unnecessary; that already the evidence in favor of filtration is overwhelming; and that velocity of flow is of contributory importance in that upon it depends renewal of fluid in contact with filtration surfaces, and the condition (permeability) of the membranes through which filtration occurs. To hold the conviction, however, that urine is first formed by pressure one must know that increase of pressure increases urine and that decrease of pressure diminishes it. The directness with which these experiments have yielded this knowledge has greatly strengthened our belief in the soundness of the filtration hypothesis.

SUMMARY

The rabbit's kidney has been perfused *in situ* with whole hirudinized rabbit's blood by a method which permits the pressure within the renal circulation to be altered without necessarily altering the total blood flow through the organ. Changes in perfusion pressure induced by splanchnic stimulation, by introduction of adrenalin and nitroglycerine, by compression of the renal vein and by stimulation of the medulla were accompanied by parallel changes in the rate of urine elimination. The results are believed to constitute direct evidence in support of the filtration hypothesis of glomerular function.

BIBLIOGRAPHY

(1) CUSHNY: The secretion of urine, 1917.
(2) GOLL: Zeitschr. f. rationelle Med., 1854, N. F. iv, 78.
(3) HERMANN: Sitzungsb. d. kaiserliche Akad. d. Wissensch. zu Wien, 1862, xlv, Abth. II, 317.

(4) USTIMOWITSCH: Arbeiten aus dem physiologischen Anstalt zu Leipsig, 1870, 198.
(5) HERMANN: Loc. cit., 346, 347, 348.
(6) HEIDENHAIN: Arch. f. mikros. Anat., 1874, x, 1.
(7) HEIDENHAIN: Hermann's Handbuch der Physiologie, 1863, V (1), 325.
(8) PANETH: Arch. f. d. gesammt. Physiol., 1886, xxxix, 515.
(9) HEIDENHAIN: Hermann's Handbuch, 1883, V (1), 324.
(10) TAMMANN: Zeitschr. f. phys. Chemie, 1896, xx, 180.
(11) RICHARDS AND PLANT: Journ. Pharm. Exper. Therap., 1915, vii, 485.
(12) RICHARDS AND DRINKER: Ibid., 467.
(13) VAN SLYKE AND CULLEN: Journ. Biol. Chem. 1914, xix, 211.
(14) FOLIN: Journ. Biol. Chem. 1912, xi, 507.
(15) FOLIN: Journ. Biol. Chem., 1914, xvii, 469.
(16) FOLIN AND DENIS: Journ. Biol. Chem., 1914, xvii, 491.
(17) COHNHEIM AND ROY: Virchow's Arch., 1883, xcii, 424.
(18) JOST: Zeitschr. f. Biol., 1914, lxiv, 441.
(19) USTIMOWITSCH: Loc. cit.
VON SCHRÖDER: Arch. f. exper. Path. u. Pharm., 1887, xxii, 39; cf.
STARLING: Journ. Physiol., 1899, xxiv, 317.
(20) STARLING: Loc. cit.
(21) MARSHALL: Journ. Pharm. Exper. Therap., 1915, vii, 157.
(22) SIEVERT: Zeitschr. f. exper. Path. u. Therap., 1909–10, vii, 532.
(23) MEYER: Arch. f. exper. Path. u. Pharm., 1902, xlvii, 426.
(24) TIGERSTEDT: Ergebn. d. Physiol., 1903, ii (2), 551–553.
KUNO: Journ. Physiol., 1915, 1, 151–152.
(25) HUBER: Amer. Journ. Anat., 1906–07, vi, 399.
(26) GERARD: Journ. de l'anat. et de physiol., 1911, xlvii, 169.
(27) GROSS: Journ. Med. Research, 1917, xxxvi, 335.
(28) RICHARDS AND SCHMIDT: To be published shortly.

URINE FORMATION IN THE PERFUSED KIDNEY

The Influence of Adrenalin on the Volume of the Perfused Kidney[1]

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The experiments reported in the previous paper (1) show that change in renal blood pressure in the perfused kidney unassociated with corresponding alteration in blood flow causes parallel change in the rate of urine elimination. They constitute, therefore, direct evidence in support of the filtration theory of urine formation. In presenting the results the same assumption is made which has been adopted by both of the two older schools of thought on kidney function, namely, that alterations in the circulation of the kidney are most effective in altering urine output by reason of changes in circulatory conditions in the glomerulus. This assumption originated in Bowman's paper, was immediately accepted by Ludwig, later by Heidenhain, and direct evidence in its support was supplied by Nussbaum.

The results with adrenalin detailed in the previous paper lend themselves especially well to further study for the purpose of attempting to identify the part of the renal vascular bed which is concerned in the increased elimination of urine following increased renal pressure.

For technical reasons no attempt was made in those experiments to record kidney volume. It is obvious that valuable information might have been obtained concerning the state of the blood vessels in it had this been successfully done, for the puzzling complications which attend the interpretation of volume records in the intact animal would have been simplified by reason of the maintained constancy of blood flow. We have tried to get this information concerning adrenalin in separate experiments designed for this purpose alone.

[1] The experiments described in this paper were reported at the twenty-ninth annual meeting of the American Physiological Society (This Journal, 1917, xlii, 592).

If adrenalin acts alike on the afferent and efferent vessel of the glomerulus then in our perfusion experiments a marked increase in intraglomerular pressure should occur. Our pump is amply able to drive the blood through the constricted vessels; narrowing of the afferent vessel would increase velocity of flow in it but would not greatly affect pressure beyond it, but narrowing of the efferent vessel would certainly cause increase in pressure in the glomerular capillaries proximal to it. If this increase were sufficient, glomerular swelling incident to it might occur sufficient to cause increase in volume of the whole kidney, despite the shrinkage of afferent and efferent vessels. If this were true then perfusion pressure should rise from constriction of afferent and efferent vessels and the kidney should swell from glomerular distention.

There is good reason for thinking that adrenalin may act upon both the afferent and efferent vessel. Elliott's generalization concerning the equivalence of adrenalin action with stimulation of sympathetic vasoconstrictor nerves is universally accepted. Smirnow has demonstrated the existence of nerve ends in the efferent vessels (2):

In der Wandung der Vasa afferentia der Knäuel färbten sich in einzelnen Fällen von verschiedener Dichtigkeit Nervengeflechte aus marklosen Fasern, und sogar in einigen der Ausfuhrungsgefässe färbten sich, namentlich durch Methylenblau, die motorischen Nervenendigungen in Form von feinen väricösen Nervenfaden, die sich wiederholt verzweigten und gewöhnlich mit Endanschwellungen auf den glatten Muskelzellen endigten, welche zuweilen in Art einer unterbrochenen Schicht in der Wandung des Vas efferens in der Nähe des Ausganges desselben aus der Bowman'schen Kapsel auftraten.

In this work excised kidneys from the rabbit and dog were placed in an oncometer and were perfused by means of the pump used in the previous experiments. Hirudinized blood from the same animal was used as perfusion fluid; no attempt was made to establish the perfusion current without interruption of circulation, though it is clearly desirable that such interruption be short. As a result of the interruption in the circulation no urine was formed in the experiments; hence expansion of the kidney from the formation of urine within it in these experiments need not be considered.

The volume recorder used was constructed according to Jerusalem and Starling (3); it was not as sensitive as a Brodie bellows. A change of 8 mm. in the level of the volume recorder was equivalent to a volume change of 1 cc. The perfusions were conducted at 37°C. Rate of perfusions was estimated by measuring venous outflow. Perfusion pressure was recorded by a Hürthle manometer. Injections of adren-

alin were made by means of a 1 cc. syringe the needle of which was thrust through the wall of the rubber tubing connecting the pump with the renal artery. Adrenalin dilutions were made with blood.

As controls similar experiments were carried out with the dog's fore-leg. Neither the intestine nor spleen is suitable for such test on account of the action of adrenalin on the intrinsic muscle of each.

The results are illustrated in figures 1, 2 and 3. In the experiment shown in figure 1, the dog's fore-leg was perfused. The injection of 0.0002 mgm. adrenalin at *A* caused perfusion pressure to rise from 40 to 48 mm. Hg.; leg volume decreased. Injection of 0.0005 mgm. adrenalin at *B* caused perfusion pressure to rise from 42 to 60 mm.; decrease of leg volume was more pronounced. When these tests with the fore-leg were finished, the kidney was excised and perfused (fig. 2). Injection of 0.001 mgm. adrenalin at *A* raised perfusion pressure from 52 to 220 mm., and kidney volume decreased. The great increase in pressure caused lessening in volume output of the pump from 15.5 to 11.4 cc. per minute. Injection of 0.0002 mgm. adrenalin at *B* raised perfusion pressure from 48 to 165 mm., but the kidney volume showed little perceptible change. At *C* and *D* the same nominal dosage was injected (it was actually less because the solution had been standing); pressure was increased at *C* from 38 to 100; at *D* from 38 to 90 mm., but in both instances the kidney volume increased. In the last three tests, rate of blood flow through the kidney was practically constant before, during and immediately after the action of the adrenalin. In both tests, *C* and *D*, the kidney swelling was quickly followed by return to normal volume. No urine issued from the kidney, hence the swelling must have been due to dilatation of vessels.

In figure 3 a similar experiment with the rabbit's kidney is shown. Injection of 0.0002 mgm. adrenalin into the perfusion stream caused such constriction of vessels that perfusion pressure increased from 70 to 130 mm. Hg.; but the kidney volume after an inconsiderable decrease showed an unmistakable swelling followed by return to normal.

The results prove a coincidence of vasoconstriction, as shown by rise of perfusion pressure, and vasodilatation as shown by increase in kidney volume. Since the conditions under which it occurred were similar to those in the previous experiments which yielded increased urine, we may interpolate this result into the records of those experiments and assume that in the kidney perfused at constant rate of blood flow adrenalin causes rise of renal blood pressure, increase of kidney volume and diuresis. The identity of the action of adrenalin and constrictor

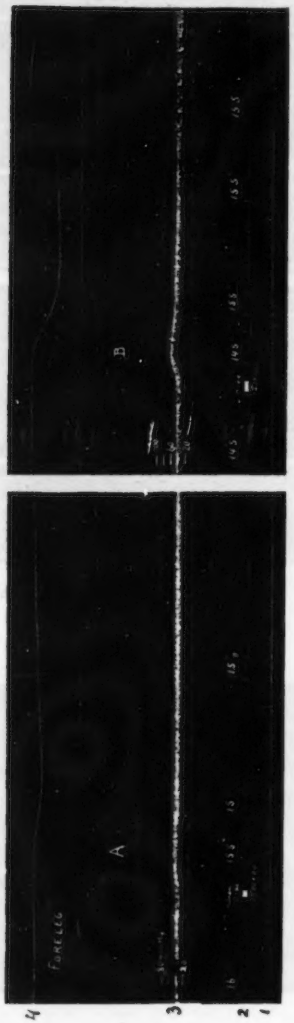

Fig. 1. Experiment of September 23, 1916. Perfusion of the dog's fore-leg with hirudinized blood at constant rate of blood flow. *1*, time in seconds; *2*, signal showing adrenalin injections and blood flow estimations (time required for 4 cc. of blood to flow from renal vein); *3*, perfusion pressure; *4*, volume of fore-leg.

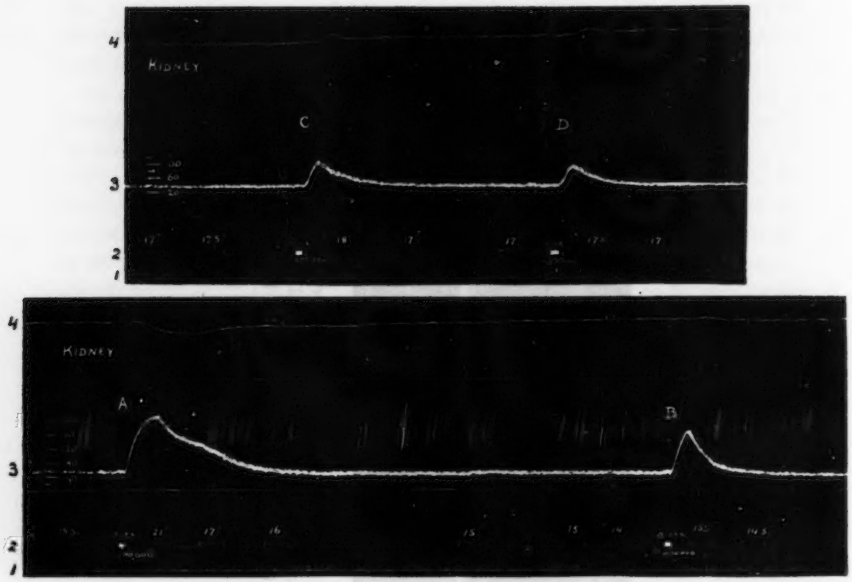

Fig. 2. Same experiment as figure 1. Perfusion of dog's kidney with hirudinized blood.

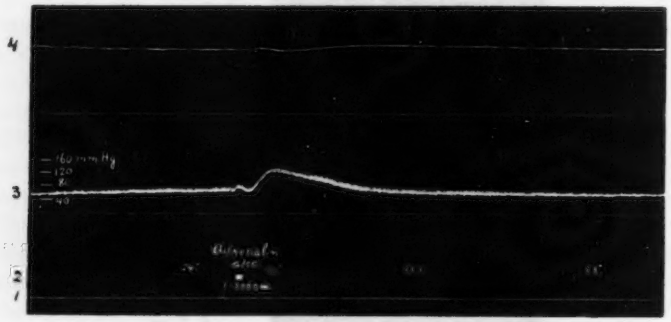

Fig. 3. Experiment of October 23, 1916. Perfusion of rabbit's kidney with hirudinized blood at constant rate of blood flow. Estimations of blood flow in curve 2 show time required for 3 cc. of blood to issue from the renal vein.

nerve stimulation permits the same assumption regarding the action of medullary stimulation and stimulation of splanchnic nerves as demonstrated in the previous experiments.

Since this coincidence of vasoconstriction and vasodilatation does not occur in the leg when its vessels are perfused in the same manner, we must attribute it to the arrangement of vessels which exists in the kidney and not in the leg. No other explanation presents itself than that already suggested, viz., that under these conditions of perfusion constriction of the afferent arteriole does not prevent access of blood and transmission of pressure to the vessels beyond; constriction of the efferent vessel must increase pressure in the glomerular capillaries. Their consequent distention caused the kidney to swell. Both the degree of kidney swelling and its time relations support this view. The fraction of kidney tissue consisting of glomeruli is small, and the existence of the glomerular capsule must lessen the effectiveness of distention of glomerular capillaries in causing the whole kidney to swell. In both the dog and rabbit the swelling of the kidney occurs later than the rise of perfusion pressure; indicating that the vasodilatation is the result of the vasoconstriction. It should be noted that in the previous experiments the onset of diuresis was similarly delayed.

This striking coincidence of vasoconstriction and vasodilatation within the kidney produced by adrenalin is therefore offered as evidence that the action of adrenalin upon the kidney includes constriction of the efferent arteriole.

Acting upon a suggestion made by Professor Cushny, an attempt was made to amplify these results by testing the effect of adrenalin in a perfusion of the frog's kidney. The experiments were hardly successful enough to warrant description. In one, however, in which the renal arteries were perfused from a reservoir via the aorta with oxygenated Ringer's solution at a pressure of 22 cm. of water and the renal portal vein at a pressure of 7.5 cm., the oncometer record showed slight shrinkage of the organ when a trace of adrenalin was added to the perfusion fluid passing through the renal portal system. This result is regarded as of value only as it directly indicates that vessels distal to the glomerulus are susceptible to the constrictor influence of adrenalin.

It is not improbable that adrenalin has the power of causing constriction of the capillaries of the tubules, and if so this might account for the result in the frog's kidney. It is inconceivable that constriction of capillaries of the tubules could be responsible for the results described

in the dog's and rabbit's kidneys. The tubular fraction of the vascular bed is too great. The result is useful in showing that adrenalin does not cause these capillaries to dilate.

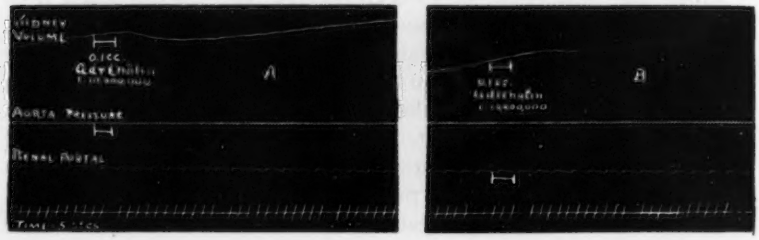

Fig. 4. Experiment of July 7, 1919. Perfusion of a frog's kidney with oxygenated Ringer's solution via the aorta at a pressure of 22 cc. of water and renal-portal vein at a pressure of 7.5 cc. of water. *A* shows the effect of a trace of adrenalin injected into the arterial perfusion fluid; *B* shows the effect of similar dose injected into the venous perfusion.

SUMMARY

When the kidney of the dog or rabbit was excised and perfused with hirudinized blood at a constant rate of blood flow, the addition of a small amount of adrenalin to the blood caused rise in perfusion pressure and swelling of the kidney. This result is interpreted as evidence that the vas efferens is constricted by adrenalin with consequent rise of intraglomerular pressure and distention of the Malpighian bodies. It is regarded as explanatory of the diuresis caused by adrenalin, splanchnic stimulation and medullary stimulation reported in the previous paper.

BIBLIOGRAPHY

(1) Richards and Plant: This Journal, 1922, lix, 144.
(2) Smirnow: Anatom. Anzeiger, 1901, xix, 353.
(3) Jerusalem and Starling: Journ. Physiol., 1910, xl, 287.

THE ACTION OF MINUTE DOSES OF ADRENALIN AND PITUITRIN ON THE KIDNEY

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Facts have been presented in the two previous papers (1) indicating that in the kidney, perfused at constant rate of blood flow, adrenalin causes rise of renal blood pressure (vasoconstriction), diuresis and increase in kidney volume. We regard the diuresis and kidney swelling as due to increased glomerular pressure, resulting from constriction of the vas efferens. The demonstration of this striking coincidence of vasoconstriction and vasodilatation within the perfused kidney has led to the belief that a similar coincidence with similar implications might be demonstrated in the intact animal. If this were possible, the view that our perfusion experiments have yielded results applicable to the interpretation of normal kidney function would be strengthened, and new light might be thrown upon the renal action of adrenalin and other substances.

The essential difference between the effects of adrenalin in the kidney, perfused as has been described, and the effects of similar dosage in the intact animal lies in the fact that in the perfusion experiments the blood containing the adrenalin is driven at a constant rate through the renal vessels regardless of their state of constriction; in the intact animal, on the other hand, the constriction of the renal vessels diminishes markedly the vascular capacity of the kidney so that renal blood flow is diminished. In the intact animal the vasoconstriction by adrenalin in the dosages used is an effective barrier to the entrance of blood; in the perfused kidney it is not.

If the belief is correct that the action of adrenalin includes constriction of both the afferent and efferent vessels; that diuresis is caused by it in the perfused kidney because constriction of the afferent vessel can not greatly lessen the supply of blood and the transmission of pressure beyond it, while constriction of the efferent vessel must cause increase in pressure in the glomerular capillaries; then, theoretically,

it should be possible to reveal events in the kidney of the intact animal, not only in harmony with the belief but actually demonstrative of its truth.

The efferent vessel has smaller caliber than the afferent; a slightly constrictor influence, exerted equally on both, will therefore, increase resistance in the efferent vessel to a greater degree than in the afferent. If a dosage of adrenalin or of any other substance capable of constricting renal arterioles, so chosen as to produce a very slight degree of action on renal vessels, is injected into the intact animal, an effective constriction of the efferent vessel should occur: as a result blood flow through the kidney should diminish; diuresis should occur from increased glomerular pressure and the kidney should swell from the distention of the glomerular capillaries.

Our attention was first directed toward the action of adrenalin. The intensity of its constrictor action throughout the body is proportional to the degree of constrictor innervation. We do not know whether or not sympathetic nerve ends are progressively more numerous toward the periphery of an arteriole. There is physiological evidence that the action of adrenalin increases toward the periphery. Oliver and Schäfer (2) observed that the arterioles contract while larger arteries swell during the rise of blood pressure caused by adrenalin; Langley (3) stated that the effect of adrenalin on the easily visible, though small, branches of the coeliac and superior mesenteric arteries appeared to be less than that on the arterioles; Barbour (4) showed that rings cut from distal parts of the renal artery gave greater contraction than rings from the proximal part; and more recently evidence has been obtained that not only the arteriole but also the capillary is involved in its constrictor action (5). Teleologically considered, if adrenalin is a means of regulating blood supply to tissues in accordance with their needs its effects would be most economically and most exquisitely exhibited if its action were greatest upon the smallest vessels. Whether or not, in the case of adrenalin, anatomical conditions other than mere size of vessels determine the most effective action at the extreme periphery of the arterial tree is not vital to the problem before us: *a priori*, there is reason to think that an arterial constrictor acting in great enough dilution upon renal vessels will produce its most effective action upon the smallest arterioles—the vasa efferentia—and that resulting rise of glomerular pressure should distend the kidney and increase urine. The demonstration of the simultaneous existence of vasoconstriction, vasodilatation and diuresis under the influence of minute

doses of constrictor substances would be strong evidence in favor of this belief. The experiments here reported were made in order to learn whether such a coincidence can be found, during the action of two arterial constrictors, adrenalin and pituitrin.[1]

METHOD. Rabbits, dogs and cats were used. They were made diuretic before the experiment—the rabbits by feeding with carrots, dogs and cats by injections of glucose solution into the stomach some hours before the experiment.

Anesthesia was produced by injecting 1 gram of urethane per kilo into the stomach, and supplementing this by ether inhalation throughout the operations.

A tracheal cannula was inserted. Arterial blood pressure was recorded by a mercury manometer attached to the carotid artery. Intravenous injections were made through a cannula in the jugular vein, using a 1-cc. syringe graduated in 1/100's. The injections were not "washed in" with additional fluid. In some experiments the animal was completely eviscerated after successive ligation of coeliac, superior and inferior mesenteric arteries and portal vein; in others, the coeliac axis was not ligated and only the jejunum, ileum and large intestine removed. The latter procedure appeared to have some advantages. The vagus and splanchnic nerves were cut. The right kidney was ligated and in some experiments excised. Both suprarenal veins were ligated. After ligature of the abdominal aorta below the origin of the left renal artery the inferior vena cava was prepared for the Barcroft-Brodie method of estimating blood flow through the kidney (6). The left kidney was put into a gutta percha oncometer, a small Brodie bellows being used to record changes in its volume. A cannula was inserted into the left ureter and urine flow from it registered by a drop recorder.

To obtain the results to be described it appears to be essential that the animal be rendered diuretic before the actual experiment and that the preparatory operations be conducted with the utmost rapidity and with the least possible degree of exposure and mechanical insult to the kidney.

RESULTS: *Adrenalin.* In preliminary experiments with rabbits we found in a certain proportion of trials that rise of arterial pressure with swelling of the kidney, could be produced by small doses of adrenalin dilutions of the order of 1:100,000. In the experiment of July

[1] Parke, Davis and Company's preparations were used.

29, 1919, this result was obtained with 0.1 and 0.2 cc. of 1:1,000,000 but the kidney swelling was inconsiderable. Fifteen milligrams of caffeine were then injected in order to dilate the kidney vessels. The oncometer showed that this occurred. Injection of adrenalin then produced greater swelling of the kidney than before. The urine drop recorder was attached and it was found that slight increase in rate of urine flow accompanied the kidney swelling from 0.3 cc. of 1:1,000,000, adrenalin (fig. 1). After control estimations of blood flow, the dose was repeated; a similar result was obtained, and in addition it was found that renal blood flow diminished slightly during the action (fig. 2). It will be noted that arterial pressure rose (80 to 84 mm.);

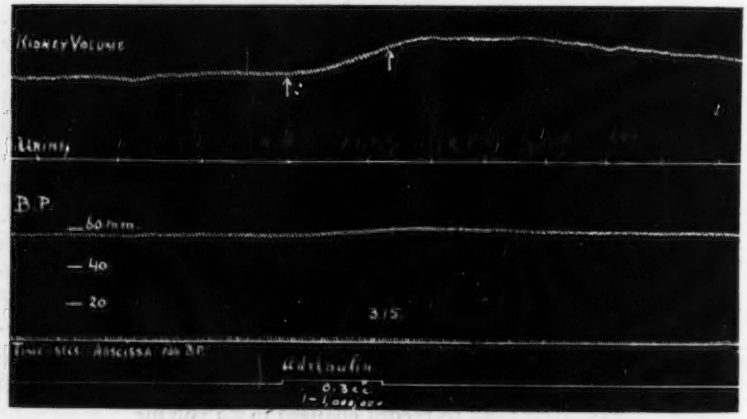

Fig. 1. Rabbit. Experiment of July 29, 1919. Adrenalin. Blood pressure, urine flow and kidney volume.

from this cause alone, renal blood flow should have increased. The evidence that slight vasoconstriction occurred within the kidney is thereby strengthened. The concomitant swelling of the kidney and increase in urine are in harmony with the assumption on the basis of which the experiment was designed.

Pituitrin. Figures 3, 4 and 5, taken from an experiment of October 31, 1919, show the results of similar tests with pituitrin in a rabbit. In figure 3 the coincidence of kidney swelling and diuresis is shown. In figures 4 and 5 the figures for renal blood flow give distinct evidence of vasoconstriction, despite the rise in arterial pressure (76–84; 75–86 mm.).

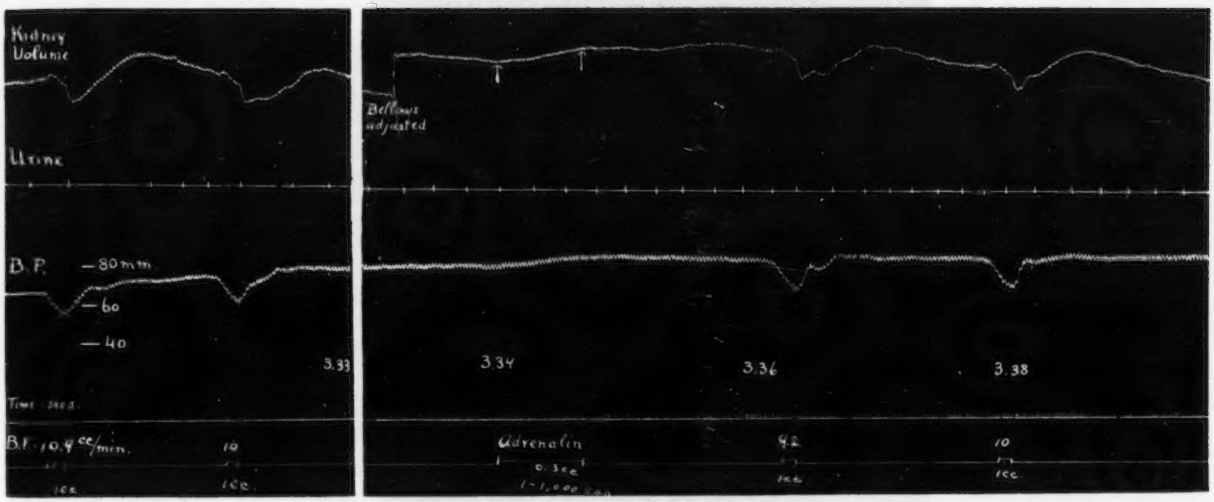

Fig. 2. Experiment of July 29, 1919. Adrenalin. Renal blood flow, blood pressure, urine flow and kidney volume.

These results were repeated in dogs with results even more impressive. In an experiment of November 26, 0.2 cc. of a 1:4 dilution of "Pituitrin S" caused blood pressure to rise from 85 to 110 mm.; urine drops before injection were 3, 5, 3, 4 and 4 per minute; after injection 3, 3, 2, 4, 4, 6, 8, 6, 4, 3; blood flow estimations before injection: 93.7, 88.2, 88.2 cc. per minute; after injection, 81, 90.9, 79, 76.9 cc. per minute. For 2 minutes after the injection the kidney contracted. From then on it expanded.

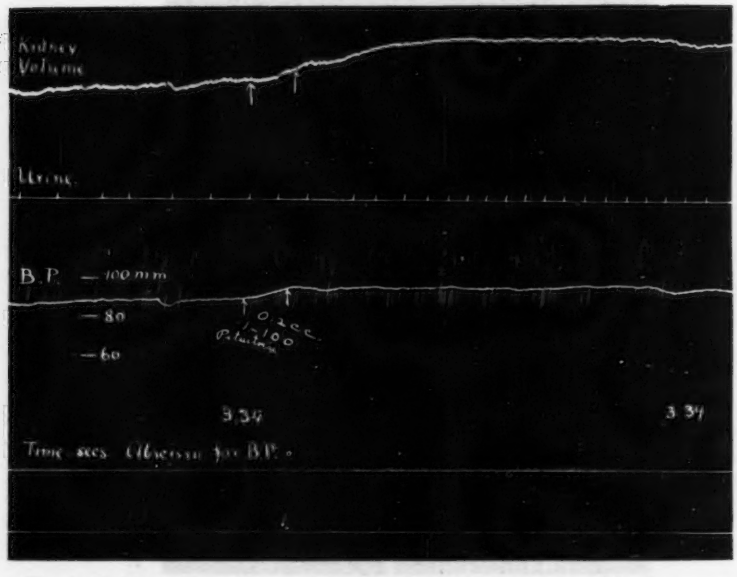

Fig. 3. Rabbit. Experiment of October 31, 1919. Pituitrin. Blood pressure, urine flow and kidney volume.

Later in the same experiment, blood pressure having fallen to 50 and urine elimination stopped, the same dose caused rise of blood pressure to 90, marked swelling of the kidney with no preliminary contraction, increase in blood flow from 42.8 to 68.3 cc. per minute. The latter result is comparable with those of Knowlton and Silverman (7) and shows that conditions within the animal may so change that the more characteristic result which we have been looking for is not to be found.

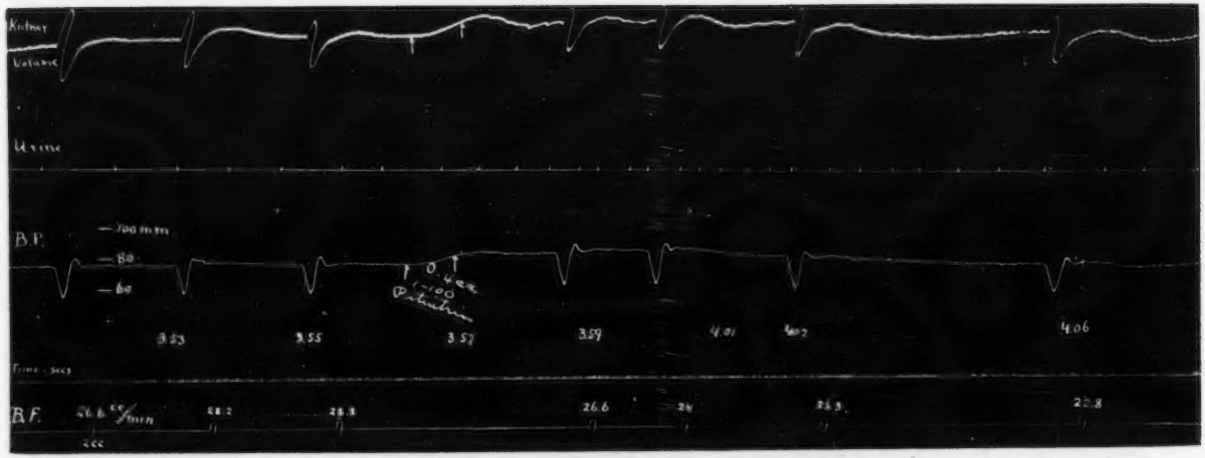

Fig. 4. Experiment of October 31, 1919. Pituitrin. Renal blood flow, blood pressure, urine flow and kidney volume.

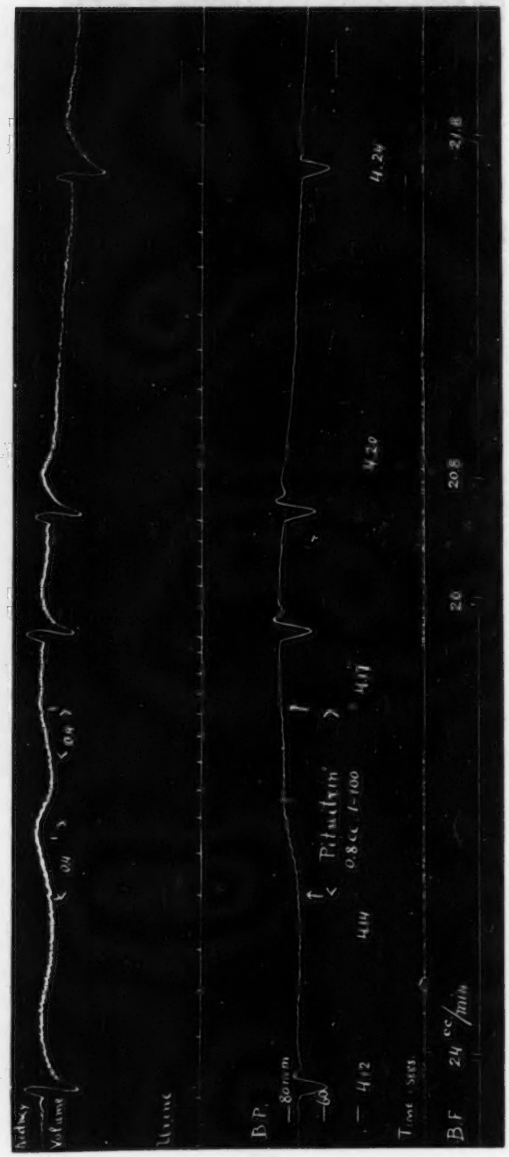

Fig. 5. Experiment of October 31, 1919. Pituitrin. Repetition of test shown in figure 4.

We have not been completely successful in obtaining these results in experiments with cats. Increase in blood pressure, swelling of the kidney and increase in urine with practically no change in blood flow is the best result we could get.

DISCUSSION

The results accord well with those of our perfusion experiments. Neither the extent of diuresis, the expansion of the kidney nor the diminution in blood flow taken in itself is impressive; their coincidence, however, is very impressive and we believe it constitutes evidence that these substances in high dilution exert a selective constriction of the efferent vessel in comparison with the afferent. We had hoped to be able to study the gaseous metabolism of the kidney during these changes, but could not. Knowlton and Silverman, however, using doses of pituitary extract which produced far greater diuresis than that observed in any of our experiments could not detect increase in it. The results provide reasonable assurance that the obvious conclusions derived from the perfusion experiments are sound.

They also throw some new light on the action of the two substances used, adrenalin and pituitrin. The diuretic action of small doses of adrenalin has been noted by Barbier and Frenkel (8), by Houghton and Merrill (9) and by Biberfeld (10). Houghton and Merrill obtained this result by intravenous injection of 0.01 mgm. into dogs (weights not specified); Biberfeld by 2 mgm. subcutaneously in rabbits. The resultant concentrations in the blood must have been of the same order of those we have employed in these experiments. Barbier and Frenkel observed both diuresis and swelling of the kidney in dogs as the general vasoconstrictor action of larger doses was passing off and in addition they reproduce one record, not unlike our figure 1, in which moderate rise of arterial blood pressure following 0.01 gram of their extract was accompanied by swelling of the kidney and slight diuresis. They did not estimate blood flow. Jost (11) has reported experiments in which adrenalin and stimulation of the splanchnic nerve were tested in rabbits during infusion of a sodium chloride solution containing theophyllin. Swelling of the kidney and slight diuresis accompanied the rise of blood pressure which was obtained. Blood flow was not estimated. Both Barbier and Frenkel and Jost interpret the oncometric record to mean dilatation of renal vessels. This explanation of the diuretic action of adrenalin is commonly accepted (12). While our results do not exclude the possibility of the production by adrenalin of a dilatation of

renal vessels in the usual sense, they show that swelling of the kidney may be accompanied by restriction of blood flow. Our perfusion experiments have supplied the reason for regarding this as evidence of constriction of the efferent vessel, and this is the explanation we wish to advance for the diuretic action of small doses.

Similar considerations apply to pituitrin. In animal experiments it most commonly causes diuresis, but decrease in urine is not infrequent (13). In man it has been successfully employed to lessen the urine elimination in diabetes insipidus (14). Its diuretic effect in cats is associated with dilatation of renal vessels and increase in blood flow (Knowlton and Silverman); but our experiments reveal the fact that a constrictor action on the efferent vessel may play a part in the diuresis from small doses. They do not show why it is that small doses may constrict while large doses dilate the renal vessels. It should be remembered in this connection that pituitary extract is a mixture of substances.

In Brodie's discussion of theories of urine formation (15), he cited as evidence against the theory of glomerular filtration the fact that in a number of experiments with diuretics he had coincidently encountered increase in kidney volume, decrease in kidney blood flow, increase in diuresis. He regarded this coincidence as completely unexplainable by the filtration hypothesis. It will be observed that this is the same combination of events as that upon which emphasis is being laid in this paper. If our experiments and their interpretation are sound, such a combination is not only not inconsistent with filtration theory, but, under certain conditions, is demanded by it. The conditions under which Brodie made the observations alluded to were not described.

The experiments under discussion have importance other than that which they possess as controls and amplifications of our perfusion experiments and as explanatory of the intricacies of the action of adrenalin and pituitrin. They yield concrete evidence that urine formation in the glomerulus is regulated not only by the gross changes in caliber of renal blood vessels which produce obvious variations in glomerular blood pressure and blood flow in the same sense, but also by finer changes in the relative caliber of the afferent and efferent vessels—changes which estimations of kidney volume or of blood flow alone do not reveal. Constrictor substances in high dilution, by acting especially on efferent vessels may increase glomerular pressure and hence urine formation at the same time that they lessen blood flow through the kidney; in larger dosage, both afferent and efferent vessels may be effectively

included in the action, with resultant decrease in glomerular pressure and blood flow and in amount of urine formed. Believing that the two substances studied in these experiments take part in the normal and emergency adjustments of bodily functions, we must also believe that the kidney, in the manner described, is included in the scope of their physiological action; and since the vascular action of adrenalin is to be regarded as equivalent to that produced by stimulation of sympathetic innervation, our results may be extended to the nervous control of glomerular pressure.

Ludwig (16) in 1856 wrote:

> Da die Zu- und Abflussröhren für die Glomeruli sowohl als auch die Nierenvenenstämme in ihren Wandungen Muskeln enthalten, so liegt die Möglichkeit vor, dass sich der Strom in den Nieren je nach den Verkürzungen dieser Muskeln ändere, selbst wenn die Herzbewegungen und die Blutfülle des ganzen Organismus ungeändert bleiben.

The ideas under discussion in this paper are implied in this expression. They are more explicitly stated by Starling (17):

> On the other hand, a dilatation of the afferent vessels and a slight constriction of the efferent vessels would cause a considerable rise of pressure in the glomerular capillaries, and a consequent increased transudation, without necessarily altering to any marked extent the total circulation of blood through the whole organ. The changes in the afferent and efferent vessels are, however, beyond our control or powers of observation, so that it is impossible to devise at the present time any crucial experiment which might decide the nature of the process occurring in the glomeruli.

While we are conscious of the deficiencies of these experiments, we believe they furnish evidence which bears upon the nature of the glomerular regulation of urine formation.

SUMMARY

Under the influence of minute doses of adrenalin and pituitrin, blood flow through the kidney may be decreased, while the kidney volume and urine elimination may be increased. Considerations are presented in support of the belief that this combination of events reveals a slight degree of constriction of the efferent vessels, with consequent increase in glomerular pressure.

The experiments are interpreted as bearing upon the problem of glomerular regulation of urine formation.

BIBLIOGRAPHY

(1) Richards and Plant: This Journal, 1922, lix, 144, 184.
(2) Oliver and Schäfer: Journ. Physiol., 1895, xviii, 230.
(3) Langley: Journ. Physiol., 1910, xxvii, 237.
(4) Barbour: Arch. f. exper. Path. u. Pharm., 1912, lxviii, 41.
(5) Langley: Loc. cit.
Cotton, Slade and Lewis: Heart, 1917, vi, 221.
Dale and Richards: Journ. Physiol., 1918, lii, 110.
Hooker: This Journal, 1920, liv, 30.
(6) Barcroft and Brodie: Journ. Physiol., 1905, xxxii, 20.
(7) Knowlton and Silverman: This Journal, 1918, xlvii, 1.
(8) Barbier and Frenkel: Journ. d. physiol. et d. pathol. générale, 1899, i, 950.
(9) Houghton and Merrill: Journ. Amer. Med. Assoc., 1908, li, 1849.
(10) Biberfeld: Arch. f. d. gesammt. Physiol., 1907, cxix, 341.
(11) Jost: Zeitschr. f. Biol., 1914, lxiv, 441.
(12) Cushny: Pharmacology and Therapeutics, 6th ed., 1918, 371.
(13) Cf. Schäfer and Herring: Phil. Trans. Royal Soc., 1908, cxcix, 1.
Motzfeldt: Journ. Exper. Med., 1917, xxv, 153.
(14) von den Velden: Berl. klin. Wochenschr., 1913, l, 2083.
(15) Brodie: The Harvey Lectures, 1909–10, v, 81; Proc. Royal Soc., B, 1913–14, lxxxvii, 571.
(16) Ludwig: Lehrbuch der Physiologie, Bd. II, 1856, 257.
(17) Starling: Principles of human physiology, 1st ed., 1912, 1277; 3d ed., 1920, 1203.

THE ACTION OF BETA RAYS OF RADIUM ON EXCITABILITY AND CONDUCTION IN THE NERVE TRUNK

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The extraordinary susceptibility of dividing cells to radiations from radium, and the practical advantages arising therefrom in the treatment of malignant growths has focused attention on the disturbances which these rays produce in mitosis, at the expense of other aspects of their physiological action. This tendency has led to difficulties, for of all vital phenomena cell division is perhaps the least comprehensible and the most unyielding to analysis in physico-chemical terms.

The fertilized ovum which has been the object of many radiological investigations, is in a sense the least differentiated of all animal cells. In contrast to this the most highly differentiated cell and that which seems farthest removed from the capacity for mitotic division, is the nerve cell; and that part of the nerve cell in which the prime function of nervous tissue, conduction, is most completely isolated, is the nerve fiber. In the nerve fiber this isolated function can be easily subjected to experimental conditions and studied as such.

The recent advances which have been made in our knowledge of the nature of the nerve impulse by Lucas and Adrian, and the insight into the underlying processes afforded by the mechanical analogies described by Lillie, make it reasonable to hope that some progress in the understanding of the physiological effects of radium rays may arise from a study of their action on the functions of the nerve trunk. This hope is strengthened particularly by the consideration that the physiological effects of these radiations must be of an electro-chemical nature, and that everything we know of the nerve impulse points to the conclusion that it is essentially an electro-chemical phenomenon.

Lazarus-Barlow and Dunbar (1) have shown that the α-rays from 7 mgm. of radium bromide (at a distance of 1 cm.) cause a lowering of the threshold of excitation in a nerve muscle preparation, an effect especially marked when the stimuli are applied to the nerve, less so when applied

directly to the muscle. The radiated preparations survive longer then the controls. They found the β- and γ-rays from their comparatively weak source to be ineffective. The interpretation of these experiments is rendered difficult because both nerve and muscle were exposed to the radiation, and as Lucas has pointed out (2), the nerve-muscle preparation contains three distinct excitable substances whose functions, though qualitatively similar, differ widely in their quantitative time relations. We have consequently simplified the experimental conditions by exposing only the nerve trunk to radiation, and examining the effects on excitation and conduction.

Method. The two gastrocnemius nerve-muscle preparations, from the same frog, dissected as nearly as possible simultaneously, were set up in moist chambers, similar to those used by Keith Lucas (3). These chambers consist essentially of vertical glass cylinders which could be flooded with Ringer's solution from below. In the middle was a rigid post with a small hole to which the knee joint could be pinned. Directly over the center of the chamber a hole was drilled in the hard rubber cover through which a thread attached to the tendinous end of the muscle could be led out, and, over a pulley, to a muscle lever. Through two other holes in this cover were introduced small glass tubes through the bottom of which platinum stimulating electrodes were sealed. The preparation being rigidly attached at the knee joint, the nerve was allowed to hang vertically and the stimulation electrodes were brought in contact with it, one on each side about 5 mm. apart. The stimulating electrodes were connected with the secondary coil of a single inductorium in such a way that the induction shock would pass through the two nerves in series, and that in each nerve the break shock should be descending, that is, with the cathode toward the muscle (fig 1). Approximately the same distance lay between the stimulating electrodes in each nerve. Under these conditions the thresholds of the two preparations were found to be very nearly the same.

One chamber was provided with a special mechanism for holding the radio-active material in readiness but shielded from the nerve-muscle preparation until such time as the radiation was to begin. It could then be placed in position for radiating the nerve, without in any way disturbing the preparation or allowing dry air to have access to it. In the cover of this chamber an additional hole was drilled through which passed a steel rod, to the lower end of which was secured an applicator, consisting of a block of lead with a hemi-cylindrical cavity in one side in which the tube of emanation was placed in a vertical position and

secured with wax. This could be raised and the applicator brought to face a lead shield secured to the under side of the cover in such a way that little or no radiation could escape from the applicator in any direction. When the radiation was to begin, this steel rod could be lowered and rotated until the applicator faced the nerve in the desired position, and thus clamped by means of a set-screw in the side of the cover.

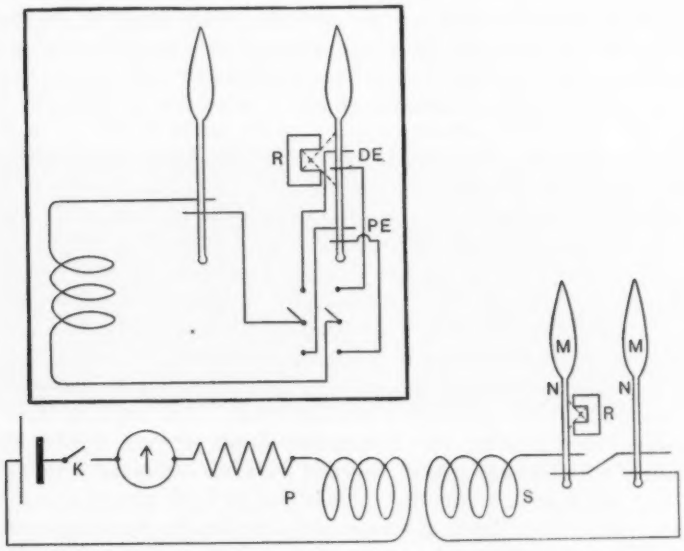

Fig. 1. Arrangement of circuits for simultaneous stimulation of radiated and control nerves. *P*, primary circuit; *K*, circuit-breaking key; *S*, secondary circuit; *MM*, muscles; *NN*, nerves; *R*, applicator with radium emanation. Inset shows secondary circuit in later experiments in which the radiated nerve could be stimulated either in the radiated region, *DE*, or promixal to it, *PE*.

The source of radiation employed was radium emanation in amounts varying in different experiments from 12 to 180 millicuries, enclosed in glass tubes approximately 1 mm. in diameter and 7 to 15 mm. in length. The walls of the tubes were thick enough to stop all the α-rays. The effects were consequently due to the β- and γ-rays, the former undoubtedly predominating because of the much greater energy absorbed from them in their passage through the tissue.

The intensity of radiation has been estimated in accordance with the formula of Wood and Prime (4), and expressed in millicurie centimeters, the intensity units employed by Redfield and Bright (5). The intensity so estimated only applies accurately to the point on the nerve directly opposite the center of the tube. This is the point of maximum radiation; above and below this point the intensity of radiation must fall off,—slowly until the points opposite the ends of the tube are reached, and more rapidly as these points are passed. The chief error in estimating intensity was due to the difficulty in determining the exact distance of the tube from the nerve, which was between 5 and 8 mm. This distance was measured by holding a millimeter scale as near to the nerve as was possible outside the moist chamber, and judging the distance by eye. The estimate could thus be made correct to within about 1 mm. The intensity employed in the different experiments varied from 31 to 685 millicurie centimeters.

For stimulating we used a Berne inductorium calibrated for break shocks in accordance with Martin's scale (6). A resistance was introduced in the primary circuit to reduce the current to about 0.1 ampere or less, and the current was recorded with an ammeter in each experiment. The primary current was broken by a key consisting of a sharp-pointed amalgamated copper rod dipping into a cup of mercury.

The muscle levers connected with the two muscles were arranged to write one above the other on a smoked drum. The usual method of recording was to start the kymograph drum moving slowly, then stimulate with break shocks at 5-second intervals beginning with a coil distance which gave a stimulus subminimal for both nerves, and moving the coil inwards 5 mm. at a time until the stimulus became maximal for both nerves. The final position of the coil was noted and the drum was stopped until the time for the next test. In this way graphic records were obtained showing both minimal and maximal stimuli for both nerves; in other words, the thresholds of the most excitable and and the least excitable fibers in each nerve. A typical series of records so obtained is shown in figure 2. The experiments were done at room temperature, about 20°C. Thermometers inserted in the moist chambers showed that they remained at practically the same temperature.

The usual procedure was to immerse the two preparations in Ringer's solution as soon as they were dissected, then set them up in the two chambers, and when lowered into position, flood each with Ringer's solution to the level of the origin of the muscle (i.e., till the whole nerve had been immersed), then let the fluid fall till it ceased to make

contact with the nerve. The moist chamber being closed from the time of flooding, no drying could occur. All was then in readiness to begin observations without disturbing either preparation. A record was then taken as described above, and this was repeated every ten or fifteen minutes until it was assured that both preparations were in a steady condition,—which usually occurred at the end of half an hour or so. Then the emanation was lowered into position to radiate the nerve, care being taken not to touch the nerve or disturb the electrode contacts of either preparation. More records were taken as above to show the thresholds and maximal contractions, first at frequent intervals (not over 5 minutes), and later at gradually increasing intervals. The observations were continued as late into the evening as was

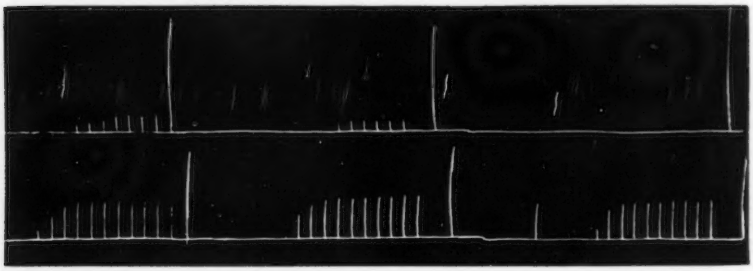

Fig. 2. Three typical series of records taken from the end of an experiment, indicating loss of function in radiated nerve. Upper line, radiated; lower line, control. The drum was moving continously during each of the three tests, the strength of stimulus being progressively increased (see text). At the end of each test the drum was stopped and synchronous ordinates were drawn.

feasible and resumed again the following morning. In some cases radiation was stopped after from one to six hours, in others it was continued throughout the experiment. Records were obtained from twenty-eight experiments with radiation.

Control experiments. Several experiments were performed in order to determine the degree of uniformity that could be relied on in the absence of radiation. Not only were the thresholds of the two nerves nearly the same in most cases, but they regularly remained approximately constant for several hours. In most control experiments, both preparations retained their vitality over night and, while showing higher thresholds the next morning, still differed from each other by a comparatively small amount.

In one experiment both preparations continued to react normally for fifty hours. The thresholds of these two preparations are plotted in figure 3 and it will be noticed that the degree of parallelism for the first forty hours was remarkable. In this instance the lead applicator was kept in the position normally used for radiating throughout the experiment, to control the possibility that any unknown influence of the lead might exert on the nerve an effect simulating that of the rays. The absence of any such effect is well demonstrated in this experiment. The usual steady behavior of the control preparation in those experiments in which radiation was employed, provided a very satisfactory basis of comparison.[1]

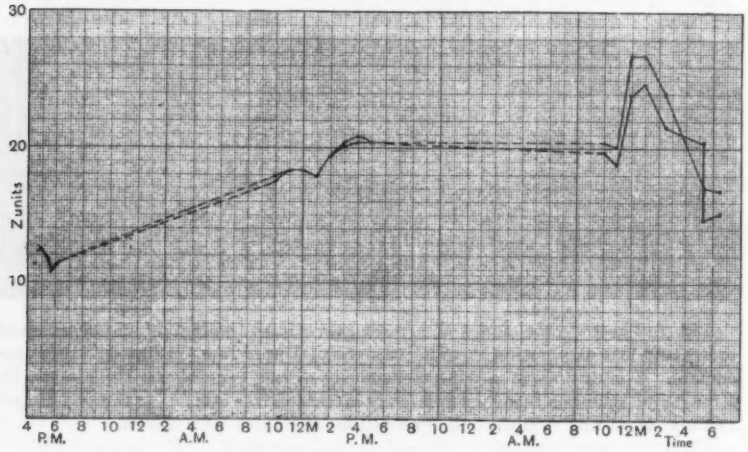

Fig. 3. Graph of control experiment. Abscissae, time; ordinates, thresholds in Z units. Dotted lines represent probable approximate course of thresholds during night when no observations were made.

Functional changes produced by radiation. Our experiments all agree in demonstrating that the ability to function disappears more rapidly in nerves exposed to strong β-radiation than in controls. That high intensities of radiation destroy the nerve more rapidly than low inten-

[1] It should be borne in mind that even a slight redistribution of the film of fluid adhering to the nerve may produce a marked change in the apparent threshold, for this will change the distribution of the lines of current flow between the stimulating electrodes. Minor changes of threshold are therefore to be expected, especially during the first minutes after flooding, and do not necessarily signify any changes in the condition of the tissue.

sities is shown by the examples in table 1. In all these examples radiation was continued till the preparation ceased to respond.

The correlation between intensity of radiation and speed of destruction is probably greater than appears in the table, since with 138 millicuries at a greater distance the radiation, though less intense at any one point than in the experiment preceding this one in the table, extended over a greater length of nerve, consequently the total blocking effect on the nerve impulse was relatively large; in this way may be explained the rapid action in this case. Even if the effect of these differences in experimental conditions could be properly evaluated it is probable that no precise relationship could be developed between the intensity of radiation and the time required to abolish function in such experiments as these; the individual variations in the different preparations would still be far too great.

TABLE 1

MILLICURIES OF EMANATION	DISTANCE FROM NERVE	INTENSITY OF RADIATION	RADIATION BEFORE FUNCTION WAS DESTROYED
	mm.		*hours*
105	3	685	1.25
111	5	350	2.75
138	6 to 8	193 to 318	2.00
85	5	291	19.0 to 20.0
64	5	200	11.0 to 20.0
34	6 to 7	58 to 75	10.5 to 18.0

It is justifiable however to indicate the difference in sensitivity between the nerve trunk and certain other tissues to the action of β-rays. In our experiments the strongest radiation which failed to abolish function had an intensity of 82 millicurie centimeters at a point on the nerve trunk where its strength was greatest, and was applied for $3\frac{1}{2}$ hours. The weakest radiation which abolished function was 31 millicurie centimeters, lasting $5\frac{1}{2}$ hours. In this case the radiated preparation ceased to respond between 22 and 25 hours after the commencement of radiation. The control was still active at the end of this time, although its threshold had risen considerably. The minimal effective dose may thus be expressed approximately as 170 millicurie centimeter hours. Redfield and Bright (5) observed marked effects on the process of membrane formation in Nereis eggs with doses as small as 7 millicurie centimeter hours, while Packard (7) observed changes in the rate of cell division in Arbacia eggs when radiated with doses of β-rays equivalent

to 2 or 3 millicurie centimeter hours. Clearly the nerve trunk is exceptionally resistant to the action of β-rays. It is little wonder that Lazarus-Barlow and Dunbar failed to obtain effects from nerve muscle preparations exposed to intensities equivalent to less than 7 millicurie centimeters.

Latent period. An interesting character of the effect of radiation on the nerve trunk is the latent period which may be demonstrated between the cessation of radiation and the time when the preparation is no longer excitable. In contrast to the experiments referred to in table 1, in many cases the radium emanation was taken away after exposure had gone on for only a few hours and while the nerve was still able to function. When this was done the threshold of the radiated preparation continued to rise till all response ceased, although the control preparation continued normal. This phenomenon suggests that the destruction of the nerve trunk by β-rays is the result of two distinct processes. The first process is some change produced directly by the radiation. It may not in itself cause any impairment in the function of the nerve fiber, but as a result of it a second process is set up which goes on even in the absence of radiation and results ultimately in the destruction of the functional power of the nerve. The time required for this second process to come to completion constitutes the latent period.

A quite analogous case has been described by Hecht (8) in connection with the stimulation of certain invertebrates by light. In these cases Hecht has shown that under the influence of light a definite photochemical reaction takes place; following this a second reaction must occur before any response is elicited from the animal, and this reaction proceeds at a rate uninfluenced by continuing the illumination. The latent period required for the development of radium and x-ray burns is no doubt due to similar series of reactions.

Table 2 shows in a rough way that the length of the latent period decreases in inverse ratio to the quantity of radiation to which the nerve trunk has been exposed. It might be taken as a criterion of the amount of damage which any dose of radiation has done to the nerve. Our series of experiments is not sufficiently extensive to show, by this means, what the optimal conditions of radiation are, but it should be possible by suitably planned experiments to show in this way the relative effects of different sorts of radiation, the comparative efficiencies of equal quantities of radiation distributed over long or short periods of time, the influence of temperature, etc.

Analysis of β-ray effect. Thus far we have spoken loosely of the β-ray effect as a failure of response in the nerve, using the rise in the threshold of stimulation as a criterion. This has served well enough for a crude description of the effect, but a more careful analysis is required.

Lucas and Adrian (9) showed that the production of a nerve impulse by an external stimulus involves two distinct processes. First the stimulating current causes, at the point where it is applied, a "local excitatory process" which appears to be of the nature of ionic concentration; then, if this process becomes sufficiently intense, it in turn sets in motion a nerve impulse or "propagated disturbance" which sweeps over the tissue. The distinction is important. Adrian has shown (10) that the propagated disturbance in each conducting unit, fiber or fibril, obeys the all-or-none law; that is, its magnitude is independent

TABLE 2

INTENSITY MILLICURIE CM.	TIME OF RADIATION	INTENSITY × TIME	LATENT PERIOD
	hours		*hours*
31	5.50	170	16 to 19
54	4.00	216	17
43	6.30	271	6 to 15
144	4.00	576	0.5 to 5
620	1.16	723	0.1
303	2.40	727	0
350	4.00	1400	0
680	4.00	2720	0

of the strength of the stimulus, and dependent only on the condition of the tissue. Thus a rise in the threshold of stimulation may mean lowered excitability at the point of stimulation, due either to increased difficulty in producing a local excitatory process or to increased difficulty in the initiation of a propagated disturbance by the local excitatory process. It may mean, on the other hand, some block in the conduction of the disturbance away from the point of stimulation. A total block of conduction in the more excitable fibers might raise the threshold up to that strength of stimulus normally required to produce a maximal response; that is, to the threshold of the least excitable fibers. On the other hand, a block in conduction involving all the fibers, but extending only a short distance from the point of stimulation, may produce an apparent rise in the threshold due to the increase in current required to produce an electrical disturbance of stimulating strength beyond the block.

With these considerations in mind, we must scrutinize the available data with a view to determining if possible whether the effect of β-radiation is one which involves excitability, or conduction of the nerve impulse, or both.

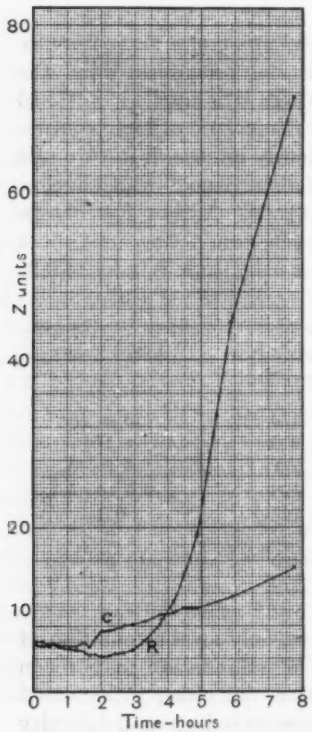

Fig. 4. Experiment showing preliminary fall of threshold, followed by rise, in radiated nerve; 85 millicuries; 5 mm. from nerve opposite point of stimulation; intensity, 291 millicurie centimeters.

In some of the earlier experiments the emanation was placed opposite the point of stimulation; thus the nerve was stimulated in the region most strongly radiated. In others the emanation was placed opposite a point between the stimulating electrodes and the muscle; thus the impulse was initiated outside the region of maximum radiation, but had to pass through it to reach the muscle. The former group might bring out effects on excitability, i.e., on the ease with which the local excitatory process can be produced or the ease with which this in turn can set up the propagated disturbance; effects which do not appear in the latter group. An effect on conduction, extinguishing the nerve impulse by gradual decrement (as is the case with narcosis) and not by complete, immediate block (as is the case with a ligature), would appear more readily in the latter group in which the whole of the strongly radiated region, instead of only part of it, lay in the path of the nerve impulse.

In some of the experiments in which the stimulus was applied in the strongly radiated part of the nerve there occurred a fall of the threshold of the radiated nerve, followed by a rise which continued till the nerve became wholly inexcitable. This preliminary fall of threshold was fairly marked in three different experiments, the threshold decreasing between one and two hours after the commencement of radiation, there being in the control threshold no corresponding fall in one of these, and a much smaller fall in the others. One of these experiments is illustrated in figure 4.

In one experiment in which the nerve was radiated between the point of stimulation and the muscle, the threshold of the radiated preparation fell notably below that of the control and continued to fall until 21 hours after the beginning of radiation. The emanation (35 millicuries placed 6 mm. from the nerve at the nearest point) was not more than 25 mm. from the point of stimulation; therefore the nerve at this point was radiated with about 7 per cent of the maximum intensity. With the exception of this experiment none of those in which the radiation was applied distal to the stimulus showed any fall in the threshold of the radiated nerve; but, as stated above, some of the other group did.

We were therefore led to suspect that radiation might at some stage of its action facilitate stimulation in the region radiated; in other words, cause a local increase in excitability. It is unreasonable to expect a fall of threshold outside the region radiated. A change in the nerve remote from the point of stimulation, but lying in the path of the nerve impulse, could not affect the response of the muscle unless it were a change which impaired conduction. Anything which would lower the threshold below normal must act at the point of stimulation. We must therefore infer that if there is any lowering of threshold more than the minor changes occurring in the controls, it must be due to such radiation as reaches the point where the stimulus is applied, unless there can be a spread of the altered state along the fibers.

Any effect on excitability would be of some theoretical interest in view of the nature of β-radiation (bombardment by free electrons) and the present theories of excitation (e.g., Nernst's theory of ionic concentration near the cathode). We decided to look for such an effect by applying two pairs of electrodes to the radiated nerve, one pair opposite the tube of emanation, and the other pair proximal to it. They were connected with a double-pole double-throw switch which rendered them interchangeable. Seven experiments were performed with this method. The results of this showed striking uniformity. Two typical experiments of this series are illustrated in figure 5. In the first of these the nerve was radiated with 111 millicuries of emanation in a tube placed 5 mm. from the nerve opposite the distal pair of electrodes. The intensity of radiation was 350 millicurie centimeters. The curves show the thresholds of the control and of the radiated nerve on stimulating through each of the two pairs of electrodes.[2] It will be noted *a*, that the threshold began to rise earlier when the stimulus was applied in the

[2] The rise in threshold in the curve *UC*, unaccompanied by any in *LC*, denotes increased resistance in the secondary circuit; this must be in the radiated nerve.

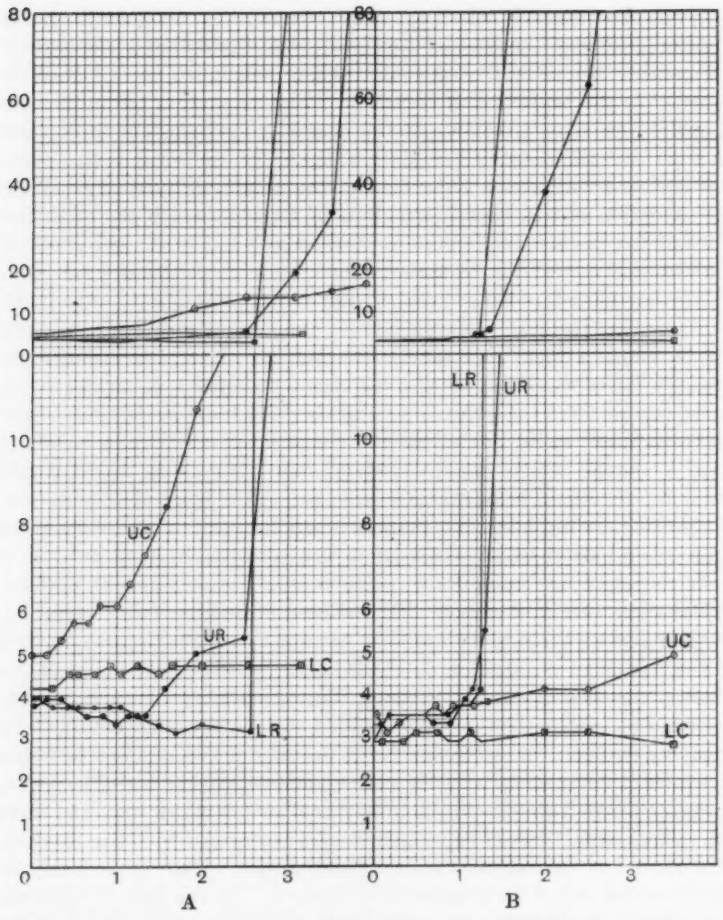

Fig. 5. Two experiments showing difference between thresholds on stimulation in radiated region and proximal to it. The lower curves are plotted on a large scale to show minor changes in initial stages of the effect; the upper curves on a small scale to show more clearly the continued rise of threshold in radiated region after conduction has ceased from promixal electrodes. *UR*, stimulus in radiated region; *LR*, stimulus proximal to radiated region; *UC*, control threshold with radiated part of radiated nerve in circuit; *LC*, control threshold with proximal part of radiated nerve in circuit. *A*, 111 millicuries; intensity 350 millicurie centimeters. *B*, 105 millicuries; intensity, 685 millicurie centimeters. Both radiated continuously till conduction ceased. Ordinates, Z units; abscissae, hours.

radiated region; but *b*, that when the rise of threshold appeared at the proximal pair of electrodes (outside) it rose very much more rapidly than it did in the radiated region.

a. The fact that the threshold rises first in the radiated region appears to signify some effect other than the disturbance of conduction. Apparently the radiation causes a local depression of excitability, a stimulus applied in the radiated region during this early stage has greater difficulty in setting up a propagated disturbance than one applied outside the radiated region, and yet conduction has not been sufficiently impaired to cause any apparent rise of threshold at the proximal electrodes.

b. After the rise of threshold at the proximal electrodes began a condition was soon reached in which no response at all could be obtained by stimulating with them, although the threshold at the distal electrodes had risen only to 50 Z units from an initial value of about 4. Out of six experiments in which observations were made frequently during the time when the changes were appearing, five agreed essentially in showing the effects just described; that is, the earlier rise of threshold with the distal electrodes and a subsequent and more abrupt rise with the proximal electrodes. In the sixth experiment both thresholds rose more gradually, but also simultaneously.

The total failure of response to stimulation through the proximal electrodes while response was still obtainable from the distal ones, is readily intelligible on the basis of a block in conduction which extinguishes the nerve impulse by gradual decrement. An impulse which must pass through the entire radiated region would be extinguished at an earlier stage of the effect than one initiated in the middle of the radiated region and only having to pass through half the region of decrement.

There might be a basis for explaining the phenomena just described without the conception of decrement, i.e., assuming that when a block is established the impulse stops abruptly upon reaching it, and without assuming any loss of excitability prior to the establishment of a total block in some of the fibers. It is conceivable that those fibers which were excited by threshold stimulation at the distal electrodes were not the same as those excited by threshold stimulation at the proximal electrodes. If this were the case we might find as the first effect of radiation a rise in threshold at the distal electrodes if the rays happened to act first on the fibers which were most excitable in this region, but no rise in threshold at the proximal electrodes. In this case, if the rays had produced a total block in any of the fibers, there should be

a diminution in the height of contraction in response to maximal stimuli at the proximal electrodes, and this should appear coincidently with the rise of threshold at the distal electrodes. Out of the five experiments showing the effects under discussion the records of three were found suitable for examination with respect to this point. Two of these showed clearly that the rise of threshold at the distal electrodes occurred before any diminution could be detected in the size of the maximal contraction in response to stimulation at the proximal electrodes, but a very slight diminution of contraction occurred a short time before the subsequent rise of threshold at the proximal electrodes. In the third experiment a slight diminution in the maximal contraction on stimulation at the proximal electrodes, appeared about the same time that the threshold began to rise at the distal electrodes. We may therefore abandon the alternative explanation, viz., that the first rise of threshold at the distal electrodes was due to an abrupt block in those fibers which were most easily excited at this point; and we may conclude that the block in conduction involves a decrement.

It appears, then, that with a proper strength of radiation a local lowering of excitability may develop before the effect on conduction has gone so far as to cause a complete block to any of the impulses coming from the proximal electrodes. It does not follow from this that the change in excitability precedes the *beginning* of the effect on conduction, for it is well established that if a nerve impluse passes a region of impaired conduction without extinction it proceeds thereafter normally. A region of decrement may be established simultaneously with the local fall of excitability, and yet escape detection because it does not suffice to extinguish the impulse in any of the fibers. In the last mentioned experiment the block in some fibers became complete at about the same time as the first rise in threshold at the point of maximum radiation.

The increase in excitability in the radiated region, already mentioned as occurring in some of the earlier experiments, was not confirmed in any of the later ones in which two pairs of electrodes were used. The lowering of threshold in the former was in no case very great, and may have been within the range of experimental error. On the other hand in three experiments it was so marked and so consistent as to make this appear unlikely. A comparison of the conditions of radiation shown in table 3 may explain why there was increased excitability in the earlier group of experiments and not in the later; with the stronger radiations it did not appear. Unfortunately the distance of the tube

from the nerve was not recorded in the last three, but since it was our standard practice to place the tube between 4 and 8 mm. from the nerve we have assumed these limits in calculating the limits to place on the intensity of radiation. Taking the probable values of intensity which would be somewhat nearer the maximum than the minimum values in the table, it will be seen that the radiation was stronger throughout the last group than in the first group, with one or possibly two exceptions. In the first experiment of the last group the radiation was probably somewhat less intense than in the third experiment of the first group. But in any case the quantity of emanation was always less in the group showing distinctly increased excitability than in the

TABLE 3

INITIAL CHANGE OF EXCITABILITY	QUANTITY	DISTANCE	INTENSITY
Increase	27	8 to 9	30 to 36
	64	5	200
	85	5	290
Doubtful increase	34	6 to 8	61 to 76
	22	7	29
	28	7	50
	180	4 to 5	530 to 740
No increase	139	6 to 8	195 to 320
	111	5	346
	105	—	150 to 470
	109	—	150 to 490
	123	—	180 to 585

group showing a decrease. Thus in the latter group, even if the intensity at the nearest point was less, the total radiation was greater,—a factor which would promote the early establishment of a block in conduction. In the third experiment of the doubtful group, in which the greatest radiation was used, the increase in excitability was very doubtful, being only slightly greater than a corresponding increase in the control, and very transient, lasting only a few minutes before it began to fall. It probably should be excluded as an experimental error.

It seems to us probable that radiation may produce a real increase in excitability, but that it is small and transient at best, and unless the intensity of radiation and perhaps other conditions be carefully chosen at proper values the effect will be obscured by the opposite effect,

i.e., lowered excitability, or by the early establishment of a block in conduction.

It is interesting to note that in one experiment the graphic records showed just before the responses ceased altogether, an all-or-none contraction in the muscle. This harmonizes well with the experiments of Adrian with narcosis.

We may summarize the analysis of the effect of β-radiation as follows: There is a lowering of excitability in the radiated region, sometimes preceded by a slight increase in excitability (probably only found with moderate strength of radiation); the lowering continues progressively until excitability ceases altogether. There is an impairment of conduction such that the nerve conducts with a decrement as in narcosis, and this also increases progressively until the block becomes complete.

Physical changes in radiated nerves. At the end of one of our first experiments, when we removed the nerves from the moist chamber, we noted that the radiated nerve was stiff throughout the region where it had been strongly radiated, whereas the control nerve was soft and pliable, as is normal. In this case we had radiated with 144 millicurie centimeters for 4 hours and at the end of 9 hours conduction had ceased. Twenty-four hours after the commencement of radiation the nerve was taken out and explored with stimulating electrodes. The muscle was found to respond normally when the nerve was stimulated distal to the stiff part where intense radiation had been applied and in which a well defined block in conduction existed. In subsequent experiments stiffening of the nerve was noted whenever the radiation was heavy. The stiffening could not be due to coagulation by heat, since the heating effect of the rays is too small to produce an appreciable rise in temperature.

In one experiment a nerve was immersed in neutral red and Ringer's solution, and radiated from a distance of 25 mm. with a tube containing 140 millicuries of emanation, thus giving a maximum radiation intensity of 22 millicurie centimeters. After being radiated for 3 hours and 10 minutes the nerve was distinctly darker in the region of maximum radiation than in the portions more remote from the tube. When placed in dilute sodium carbonate solution the strongly radiated portion faded more slowly than the rest of the nerve.

After several of our experiments with radiation strong enough to produce stiffening, the nerves were fixed with Müllers fluid, and stained

by the Marchi method.[3] Microscopic examination of the sections showed that degeneration of the myelin sheath of the nerve has begun at certain scattered points where droplets of fat are found deeply stained. Only in a few places are these fat drops collected enough to give the typical beaded appearance of definite degeneration. Some

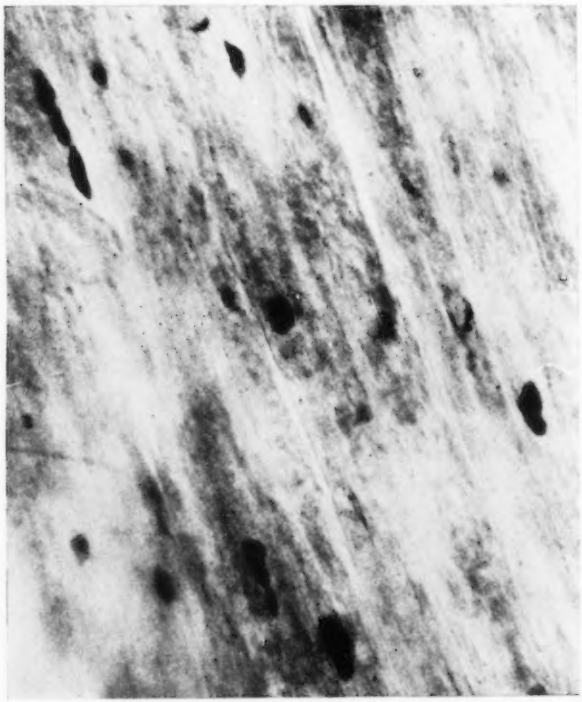

Fig. 6. Microphotograph; longitudinal section of radiated nerve, showing fatty degeneration. Marchi method.

fibers seem to be damaged more than others, and in none is the degeneration complete. Figure 6 shows a microscopic field with an unusually large amount of degenerative change but otherwise quite

[3]We wish to acknowledge our indebtedness to Dr. Annie E. Taft for assistance in making the preparations and to Dr. Stanley Cobb for examining them and making a report on which the accompanying description is based, and to Dr. N. C. Foot and Miss A. Hopkins for taking the microphotographs.

typical. The nerve had been radiated for 2 hours and 25 minutes with 120 millicuries of emanation contained in a tube 7 mm. long placed at a distance of 6 mm., the maximum intensity thus being 303 millicurie centimeters. The threshold of the preparation had commenced to rise after 2 hours of radiation and at the end of 3 hours conduction had ceased, and the nerve was found to be quite stiff over a length of 10 mm. where radiation had been most intense.

SUMMARY

1. Intense radiation with β-rays destroys the functional capacity of a nerve trunk. To produce an appreciable change the radiation must be much more intense than is required to produce marked disturbances in dividing cells or ova. Cells in mitotic division are generally believed to be especially sensitive to radiation. In view of this, the relatively high resistance of nerve fibers to radiation is somewhat noteworthy, since of all living tissues they are perhaps the farthest removed from the capacity for mitosis.

2. In the nerve trunk there may be a marked latent period between the termination of radiation and the loss of function. The latent period decreases in length as the quantity of radiation increases.

3. The disturbance of function resulting from radiation is one which involves both conduction and excitability. The effect on conduction is similar to that of a narcotic in that, in the early stages at least, conduction occurs with a decrement, so that an impulse set up in the middle of the impaired region will be conducted through the remainder of it, while one set up outside of this region will fail to pass through the whole of it.

4. Effects on excitability are shown when the stimulus is applied in the radiated region. In some experiments with an intensity of radiation so moderate as to produce the disturbance very gradually, a slight increase in excitability was found, followed by a fall. In the majority of the experiments, especially in those in which the radiation was more intense, the first and only effect of the rays was to decrease excitability and this decrease usually occurred before conduction had been so far impaired as to establish a complete block to any impulses set up proximal to the radiated region.

5. Correlated with the loss of function in the nerve trunk is a stiffening of the tissue, and this appears to be more marked the greater the radiation. Microscopic examination reveals fatty degeneration of the myelin sheath.

We wish to thank Prof. William Duane for his coöperation in placing a supply of radium emanation belonging to the Cancer Commission of Harvard University at our disposal.

BIBLIOGRAPHY

(1) Lazarus-Barlow and Dunbar: Arch. Middlesex Hosp., 1913, xxx, 17.
(2) Lucas: Journ. Physiol., 1907, xxxvi, 113.
(3) Lucas: Journ. Physiol., 1909, xxxix, 207.
(4) Wood and Prime: Ann. Surg., 1915, lxii, 751.
(5) Redfield and Bright: This Journal, 1918, xlv, 374.
(6) Martin: Measurement of induction shocks, New York, 1912.
(7) Packard: Journ. Exper. Zoöl., 1916, xxi, 199.
(8) Hecht: Journ. Gen. Physiol., 1919, i, 657.
(9) Adrian and Lucas: Journ. Physiol., 1912, xliv, 68.
(10) Adrian: Journ. Physiol., 1914, xlvii, 460.

THE EFFECTS OF ADRENAL FEEDING UPON THE IODIN CONTENT OF THE THYROID GLAND

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In previous communications it was noted that certain derivatives of the adrenal gland showed rather marked inhibitory effects upon the secretory activity of the stomach (1) and pancreas (2). Of these derivatives of the (beef) adrenal glands, the nucleoprotein material and an alcohol extract and the adrenal "residue" exhibited about equal activity. The adrenal "residue" is a term we have used to designate the final filtrate from an aqueous extract of the gland after the removal of the nucleoproteins with acetic acid, and then the coagulable acid and alkali albumens. The resultant filtrate, having been boiled at least twice, represents a slightly hydrolyzed preparation. It is rich in an epinephrin-like substance while the adrenal nucleoproteins contain only faint traces of this material. When administered hypodermically to a dog both the adrenal nucleoproteins and the adrenal residue exhibit about the same amount of gastric inhibition. These effects are best demonstrated after the administration of a thyroid "residue" made like that from the adrenal glands (1).

Alcohol extracts of the thyroid and adrenal glands show a similar contrasting visceral stimulation and inhibition but are not quite as active as the hydrolyzed residues. Commercial adrenalin showed some inhibitory effect upon the stomach, but very much less than the adrenal nucleoproteins or the adrenal residue.

It was therefore decided to ascertain whether any demonstrable effect could be produced by long-continued feeding of adrenal derivatives upon the iodin content of the dog's thyroid. Both glands of all animals used in other experiments were first examined, weighed and tested for iodin. In the considerable number of glands thus studied there were found only slight variations, though rarely the gland on one side might appear larger than on the other. If of the same size, the

iodin content was practically the same. A considerable number of animals (used in other experiments) was next treated by extirpation of one thyroid lobe, and then from 1 to 6 months later by extirpation of the second lobe. The comparison of the iodin content showed regularly a gain of about 20 per cent to 40 per cent in the second or retained lobe. The maximum gain seemed to be reached at the end of 4 or 5 weeks. There was no noticeable change in the total (fresh) size or weight of the organ. In some preliminary tests the adrenal derivatives were administered hypodermically, but the adrenal residue was thus found to produce vomiting and extreme prostration, and the repetition of the dose was often fatal. Even under these conditions, with the animal under treatment only from 3 to 10 days, the gain in the iodin content of the second lobe of the thyroid sometimes amounted to considerably over 100 per cent. But it seemed that the possible medicinal or therapeutic effect could be more fairly ascertained by a less drastic and longer continued administration of the adrenal materials mixed in the food. This consisted, for all the animals, of crackermeal, casein and water in sufficient amount to maintain the weight and metabolism at a constant level.[1] The iodin was determined by the Riggs method (3), and in the experiments reported below, by the Kendall method (4). The same individual made all of these determinations, thus reducing the errors of the personal equation to a minimum, and it is worth noting that the differences in the results between the Riggs and Kendall methods were practically negligible. The adrenal derivatives tested were the crystals of adrenalin (P., D. & Co.) and the adrenal nucleoproteins and the adrenal residue.[2] The daily dosage was 0.016 gram of adrenalin with an amount of the adrenal residue which contained approximately the same quantity of epinephrin as determined by Folin's colorimetric method (5), and 4 grams of the adrenal nucleoprotein material. The latter contains only traces of epinephrin-like material, and the 4 grams constitute, consequently, a rather arbitrary amount. All but one of

[1] Dietary of 6.5 kgm. dog:

	grams	calories
Crackermeal	60	240
Evaporated milk	30	40
Commercial casein	25	75
Commercial lard	5	45
Bone ash	10	00
Total	130	400
Water	500 daily	

[2] Supplied by Schieffelin & Co.

TABLE 1

ANIMAL NUMBER	MATERIAL FED	DURATION OF FEEDING	AMOUNT OF MATERIAL FED DAILY	EPINEPHRIN CONTENT	GLAND I REMOVED BEFORE FEEDING EXPERIMENT		GLAND II REMOVED AFTER FEEDING EXPERIMENT		IODINE INCREASE IN GLAND II OVER GLAND I		AVERAGE
					Weight of gland	Milligrams of I per gram gland	Weight of gland	Milligrams of I per gram gland	Milligrams of I per gram gland	Percentage increase	
		days		*grams*	*grams*		*grams*				
I	Adrenal residue	45	4.0 cc.	0.016	0.1970	2.03	0.3360	3.64	1.61	79.3	70.4
II	Adrenal residue	45	4.0 cc.	0.016	0.1990	2.00	0.2244	3.29	1.29	64.5	
III	Adrenal residue	45	4.0 cc.	0.016	0.2662	2.25	0.2530	3.77	1.52	67.5	
IV	Adrenalin crystals, P. D. & Co.	45	0.016 gm	0.016	0.2660	2.82	0.2580	3.96	1.14	40.7	46.6
V	Adrenalin crystals, P. D. & Co.	45	0.016 gm	0.016	0.3780	3.01	0.3468	4.47	1.46	48.5	
VI	Adrenalin crystals, P. D. & Co.	45	0.016 gm	0.016	0.1480	3.42	0.2120	4.81	1.39	40.6	
VII	Adrenal nucleo-protein	45	4.0 gms.	Traces	0.2186	3.01	0.2636	4.96	1.95	64.7	50.7
VIII	Adrenal nucleo-protein	37	4.0 gms.	Traces	0.2442	3.07	0.1250	4.40	1.33	43.3	
IX	Adrenal uncleo-protein	45	4.0 gms.	Traces	0.1183	4.15	0.1684	5.98	1.83	44.0	
X	Control	45			0.2552	1.76	0.2748	2.54	0.78	44.3	39.6
XI	Control	45			0.1818	3.20	0.1110	4.20	1.00	31.2	
XII	Control	45			0.1956	2.81	0.1260	4.01	1.20	42.7	

the dogs were given these adrenal derivatives in their standard diet for 45 days. No. VIII was accidentally killed at the end of 37 days. The table given below is self explanatory.

As remarked above, both glands in all the animals were inspected at the first (partial) thyroidectomy, and only those animals were retained in which the glands seemed of equal size. It will be noted that the glands removed at the conclusion of the experiment showed both gains and losses in the total fresh weight. The glands are, of course, very small and part of this gain or loss may be due to the better or worse trimming of the attached blood vessels and fascia, or to the greater or less amount of contained blood. But even when the gland seemed to have lost weight there was always a gain in iodin. This showed the maximum average in the dogs fed the adrenal residue. The adrenal nucleoprotein material, which contains only faint traces of epinephrin, certainly had no less effect than the pure adrenalin crystals, and the latter only showed a slight gain over the controls or untreated animals.

Preliminary to these experiments, and to ascertain whether they were worth while, a couple of dogs were tested in the same way but without standardization of the dosage. One animal received the adrenal nucleoprotein material, and the other the adrenal residue, both in rather large amounts. A third dog was retained as a control. At the end of 35 days, examination of the retained lobe of the thyroid showed in the animal fed the adrenal residue a gain of 69 per cent in iodin, and in the one fed the nucleoproteins a gain of 40 per cent, while the control showed a gain of only 12 per cent. With the exception of the control animal, which became mangy, these figures correspond quite closely.

An attempt to administer hypodermically an alcohol extract of the adrenal gland (made by extracting the gland with alcohol, removing the alcohol by evaporation and then adding normal salt solution) in comparison with the adrenal residue hypodermically, resulted in early death. The retained lobe of the animal which received the alcohol extract at the end of 10 days, however, showed a gain of 82 per cent in iodin while that which received the adrenal residue showed at the end of only 6 days a gain of 44 per cent. These preliminary tests are mentioned because a series of three animals is very small to indicate any definite conclusions. But the final small and carefully regulated experiments are so closely similar in their results to the rough tests that there seems to be demonstrated a direct influence of adrenal feeding upon the thyroid gland.

Epinephrin, or adrenalin, generally believed to represent the active principle, produces much less effect than the nucleoprotein material from the entire gland. Indeed, the iodin gain after the administration of adrenalin (Parke, Davis & Co.) is so slight as compared with the controls, that it might come within the limits of error and actually indicate no effect or a negative result.

The adrenal residue (Schieffelin & Co,), however, in all the tests, and especially when administered either by mouth or hypodermically seemed to produce an unmistakable and marked gain in the thyroid's iodin.

CONCLUSIONS

1. A somewhat hydrolyzed aqueous extract of the entire beef adrenal gland known as the adrenal residue, when fed by mouth, can produce in 45 days a gain in the iodin content of the dog's thyroid gland amounting on the average to 70.4 per cent.

2. The adrenal nucleoprotein material obtained from the entire beef adrenal gland, when fed in large dosage, shows an average increase of 50.7 per cent of the iodin content.

3. An amount of crystals of adrenalin (Parke, Davis & Co.) which is closely equivalent in amount to the epinephrin-like material of the adrenal residue produces little if any gain in the iodin content of the dog's thyroid.

4. An extract of the entire gland containing something more than pure epinephrin has thus been demonstrated to have a direct effect upon the thyroid gland.

BIBLIOGRAPHY

(1) Rogers, Rahe and Ablabadian: This Journal, 1919, xlviii, 79.
(2) Rogers, Rahe, Fawcett and Hackett: This Journal, 1916, xl, 12.
(3) Riggs: Journ. Amer. Chem. Soc., 1909, xxxi, 710.
(4) Johns and Hendrix: Journ. Biol. Chem., 1914, xix, 25.
(5) Folin, Cannon and Denis: Journ. Biol. Chem., 1913, xiii, 477.

THE EFFECT UPON THE COLD-BLOODED HEART OF CHANGES IN THE IONIC CONTENT OF THE PERFUSATE

I. Upon the Normal Mechanism

II. Upon the Arrhythmias

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The following investigation was made during the winter and spring of 1921 in an attempt to analyze the various types of arrhythmias experimentally produced in the cold-blooded heart, more especially their association with changes in the ionic content of the perfusing fluid. Preliminary to this the effects of such changes upon the mechanical and electrical phases of the activity of the turtle's heart were studied. When this first portion of the study was nearly completed the article of Daly and Clark (1) appeared, which covered this phase of the subject. While the results of our investigation were in the main confirmatory of Daly and Clark, our method differed fundamentally.

Method. The terrapin's heart (Chrysemys picta) is particularly well adapted to such experimentation since, as Lewis (2) has pointed out, a specialized conducting mechanism is wanting in the ventricle. Changes in conduction of the excitatory process must therefore be taken as due to alterations in the conductivity of the muscle itself.

Medium-sized terrapin were pithed and the plastron removed, as far as possible without hemorrhage. A small cannula with a 7.5 cm. "chimney" was inserted in the superior vena cava and an outflow established through the aorta. Perfusion pressure was regulated by means of Mariotte bottles and maintained at a level just sufficient to cause dilatation of the auricles in diastole and to allow adequate contraction in systole.

Ringer's solution was freshly prepared before each experiment according to the following formula:

	Per cent
NaCl	0.75
KCl	0.015
$CaCl_2$	0.020
$NaHCO_3$	0.015

The pH of the solution was determined by adding 5 drops of a 0.01 per cent solution of phenolsulphonephthalein to 3 cc. of the Ringer's solution and comparing with standard tubes. Such a formula as the above resulted in a solution with a pH of 7.8 to 8.0. The reaction of the perfusion fluid was adjusted by adding N/10 HCl and testing with the indicator as described until the desired pH was reached.

By means of threads attached to the surface musculature and connected with light muscle levers, the right auricle and ventricle were made to record upon a smoked drum. The amplitude of mechanical contraction was the only measurement made upon these records. The mechanical and electrical records were synchronized by an electric timer.

In taking the electrocardiographic records a standard lead was used throughout; small non-polarizable electrodes of kaolin paste containing a saturated solution of copper sulphate were placed in such relation to the heart that they gave the desired axial lead corresponding to lead II of the Einthoven nomenclature. The basal electrode was applied either upon the pectoral girdle 3 cm. to the right of the median line or upon the surface of the body just above and to the right of the right auricle. The apical elctrode was applied on the peritoneal surface to the left and caudal to the frenulum. Except where otherwise stated the string was standardized for a deflection of 1 cm. per millivolt.

Daly and Clark eliminated the natural stimulus by means of a Stannius ligature and maintained a constant frequency by stimulation with break induction shocks applied on the auricle. We have depended throughout upon the normal stimulus.

The following intervals were measured in each record:

1. R-R, representing the rate of stimulus production at the pacemaker.
2. P-R, giving the rate of conduction from auricle to ventricle.
3. QRS, the rate of invasion of the excitatory process in the ventricle.
4. R-T, as representing the duration of ventricular systole. In addition the amplitude of R, S and T and the duration of T were measured. In the later experiments the amplitude of the mechanical contraction of the auricle and ventricle was added to the above data.

PART I. NORMAL RHYTHMS

Experimental: 1. Control. Preparations were set up first without using an artificial perfusate and later with normal Ringer's solution and records taken every half-hour for 6 hours. No significant changes in the intervals as measured were observed. The accompanying table represents 3 typical examples.

Similarly, records taken before and after opening the pericardium showed no marked alterations in frequency of rate or in conduction time.

TABLE 1

	R-R	P-R	QRS	R-T	AMP. T	R
Experiment 4. Normal circulation uninterrupted						
4:30 p.m.	1.40	0.40	0.12	0.80	+9.0	18.0
7:30 p.m.	1.44	0.44	0.12	0.76	+4.5	19.0
10:00 p.m.	1.56	0.48	0.16	0.80	+3.0	22.0
Experiment 5. Normal circulation uninterrupted						
2:10 p.m.	1.68	0.52	0.16	0.92	−6.0	17.5
5:10 p.m.	1.56	0.48	0.14	0.80	−5.0	13.5
7:25 p.m.	1.72	0.52	0.16	0.86	−5.0	13.0
Experiment 6. Normal Ringer pH 7.4						
1:30 p.m.	1.72	0.56	0.12	1.16	−5.0	13-14
7:00 p.m.	1.70	0.56	0.12	0.96		16.0

2. Hypertonicity. A sufficient quantity of a 5 per cent solution of glucose was added to the perfusate to produce an osmotic pressure double that of the normal Ringer's solution. The effect of such hypertonicity is well shown in the accompanying table from experiment 14.

Hypertonicity, then, produces an increase in the rate of stimulus production, in the rate of auriculo-ventricular conduction, shortens the period of invasion in the ventricle and the duration of ventricular systole.

3. NaCl. a. Decrease. In the study of the effect of diminution in the concentration of NaCl hypotonicity was eliminated by the addition of glucose in quantity sufficient to make the mixture isotonic with normal Ringer's solution.

By reducing the concentration of NaCl the rate of stimulus production is very much decreased and its regularity may be interfered

with, conduction at the auriculo-ventricular node is greatly retarded, as is the rate of spread. The duration of ventricular systole, on the other hand, is diminished.

b. Increase. Entirely distinct from the action of hypertonicity so produced, the increase of the concentration of NaCl in Ringer's solution causes marked changes in the electrocardiogram of the turtle.

TABLE 2

Experiment 14. Normal Ringer pH 7.4

	R-R	P-R	QRS	R-T	AMP. T	DUR. T	R
11:40	2.68	0.68	0.24	1.50	−2.0 +3.0	0.16	21.5
11:42	Change to hypertonic Ringer						
11:58	2.54	0.50	0.16	1.30	−3.5 +1.0	0.24	10.0
12:00	Change to normal Ringer						
12:15	2.84	0.76	0.22	1.66	+5.0	0.40	19.0

TABLE 3

Experiment 12. Normal Ringer pH 7.4

	R-R	P-R	QRS	R-T	AMP. T	DUR. T	R
12:30	1.52	0.36	0.10	1.00	−12.0	0.20	15
12:35	Change to Ringer NaCl 0.4 per cent—made isotonic with glucose						
12:40	1.62	0.38	0.12		−4.0	0.16	10-13
12:45	1.98	0.60	0.14	0.80			7
12:50	2.54	0.52	0.15	Sinus irregularity			
12:53	Change to normal Ringer						
1:03	1.58	0.40	0.12	0.92	+5.0		8
1:19	1.62	0.40	0.10	1.00	+3.5	0.20	11

An excess of NaCl has an effect similar to a diminution of the same upon the rate of stimulus production and of auriculo-ventricular conduction. Both are distinctly impaired and there is a tendency to irregularity of stimulus production. With regard to the action upon the spread of the excitatory process and the duration of ventricular systole both are increased by an excess of NaCl in the perfusate.

NaCl alone. The effect of a change from normal Ringer's solution to a 0.75 per cent NaCl solution was studied with the results shown in

the accompanying table. All the phases of the electrical activity are retarded.

TABLE 4

	R-R	P-R	QRS	R-T	AMP. T	DUR. T	R
Experiment 12. Normal Ringer pH 7.4							
2:25	1.79	0.58	0.16	1.10	+1.0	0.10	34
2:27	Ringer 1.5 per cent NaCl						
2:53	2.02	0.72	0.12	1.28	−3.0	0.16	5
2:55	2.00	0.72	0.12	1.28	−10.0	0.16	25
3:07	2.04	0.72	0.12	1.30	−11.0	0.18	28
3:10	Normal Ringer 7.4						
3:27	1.70	0.56	0.14	1.10	−21.0	0.20	27
Experiment 21. Normal Ringer 7.4							
3:32	1.76	0.56	0.16	1.08	−8.0	0.28	18
3:40	Normal Ringer 2.0 per cent NaCl 7.4						
3:48	2.44	0.64	0.18	1.60	−1.0	0.10	17
	4.14	0.64	0.16	1.44			18
3:52	2.84	0.68	0.16	1.60	+3.0	0.14	22
	*3.80	0.68	0.16	1.50			22
3:56	2.78	0.76	0.18	1.68			
	*4.92	0.76	0.16	1.64	+3.5	0.20	21
4:00	Normal Ringer 7.4						
4:15	1.76	0.64	0.16	1.08	−8.0	0.30	16

* Sinus irregularity.

TABLE 5

Experiment 21. Normal Ringer pH 7.4

	R-R	P-R	QRS	R-T	AMP. T	DUR. T	R
3:03	1.70	0.62	0.16	1.08	−7	0.24	24
3:06	NaCl 0.75 per cent 7.4						
3:09	2.28	0.80	0.22	1.80	+2	0.36	4
3:13	Normal Ringer 7.4						
3:32	1.76	0.56	0.16	1.08	−8	0.28	18

These results may be taken as partly due to the relative excess of NaCl and in part to the absence of KCl and $CaCl_2$.

KCl. a. Decrease. Diminution in the concentration of KCl caused a decrease in the rate of the pacemaker, a decrease in auriculo-ventricular

conduction and in the rate of spread in the ventricle. Ventricular systole was prolonged. Extrasystoles and varying degrees of block occurred commonly under these conditions. Mechanically the tonus of the ventricle was very much increased.

TABLE 6

	R-R	P-R	QRS	R-T	Q-R	R	AMP. T	DUR. T	A HMR	V HMR
Experiment 29. Normal Ringer pH 7.4										
3:30	1.64	0.60	0.16	1.08	0.08	30	−10	0.40	52	67
3:32	Ringer 0.005 per cent KCl 7.4									
3:42	1.74	0.70	0.18	1.10	0.12	8	−5	0.20	45	60
3:55	Normal Ringer 7.4									
4:03	1.64	0.60	0.16	1.10	0.10	8	−7	0.28	58	60
Experiment 30. Normal Ringer 7.4										
3:58	1.72	0.64	0.12	0.86	0.08	13	−1	0.08	48	53
4:00	Ringer 0.0025 per cent KCl 7.4									
4:10	1.56	0.64	0.16	1.06	0.12	16	−1	0.10	50	55
	*2.44					20	+2			
4:12	Normal Ringer 7.4									
4:25	1.56	0.64	0.14	0.96	0.10	25	−1	0.10	52	55
	*2.08						+2			

* Sinus irregularity.

TABLE 7

Experiment 15. Normal Ringer pH 7.4

	R-R	P-R	QRS	R-T	AMP. T	DUR. T	R
1:33	1.72	0.50	0.12	1.00	+2.0	0.04	13
1:48	1.86	0.54	0.12	1.10	+2.5	0.08	14
1:53	Change to Ringer 0.06 per cent KCl						
1:54	1.80	0.64	0.12	0.80	−2.0	0.08	11
2:00	Heart markedly dilated. Circulatory failure						

b. Increase. The effects of raising the concentration of KCl to twice or even four times that of normal Ringer's solution are as follows:

The rate of stimulus production is decreased, auriculo-ventricular conduction impaired, and the duration of the systolic phase in the ventricle diminished. The rate of spread of the wave of excitation in the ventricle remains unaffected. In every experiment where KCl was

present in excess the ventricular tonus was very much decreased and continued perfusion with such a solution brought about standstill in diastole.

$CaCl_2$. a. Decrease. When Ringer's solution without $CaCl_2$ was perfused the amplitude of both mechanical and electrical records was diminished. The heart rate was retarded and the conduction at the auriculo-ventricular junction impaired. The initial spread in the ventricle underwent no significant change. Ventricular systole was somewhat prolonged.

b. Increase. An increase in the $CaCl_2$ in Ringer's solution to double and treble the normal concentration produced an initial augmentation of the amplitude of both mechanical and electrical records.

Thus it appears that $CaCl_2$ in excess of the normal concentration tends to diminish the interval of stimulus production, to facilitate conduction at the auriculo-ventricular node and markedly to increase the rate at which the wave passes off the ventricle. The rate of spread over the ventricle, as visualized in the QRS complex, is retarded.

The antagonism of $CaCl_2$ and KCl. The mechanism of this antagonism is very well illustrated in the accompanying experiment. A heart beating rhythmically with a perfusate of normal Ringer's solution was perfused with a Ringer's solution containing 0.065 per cent KCl. Circulatory failure resulted in 7 minutes. The rhythmic and previously efficient ventricular contractions gave place to a wormlike movement of the ventricular musculature which was powerless to expel any fluid through the outflow cannula.

Three minutes later a perfusate was introduced consisting of Ringer's solution containing a similar amount of KCl and a quantitatively equal excess of $CaCl_2$. In 7 minutes the ventricle had resumed powerful rhythmical contractions at, however, a much faster rate. Return to the Ringer's solution containing the excess of KCl above resulted in the reappearance of the inefficient wormlike contractions. The normal mechanism was restored with normal Ringer.

The addition of the excess $CaCl_2$ had apparently a threefold effect. It hastened impulse formation, decreased the rate of spread in the ventricle and greatly shortened ventricular systole.

$$\left.\begin{matrix} H^+ \\ OH^- \end{matrix}\right\} \textit{Ions}$$

A pH of 7.4 was taken as the normal and variations were made to either side. In no case, however, was a pH used less alkaline than 7.1

TABLE 8

	R-R	P-R	QRS	R-T	AMP. T	DUR. T	R
			Experiment 25. Normal Ringer pH 7.4				
3:18	1.18	0.44	0.14	0.80	+1.0	0.08	5
3:19	Change to $CaCl_2$-free Ringer						
3:28	1.28	0.82	0.16	1.06	−1.0	0.06	4
3:32	Change to normal Ringer						
3:42	1.10	0.42	0.14	0.74	−1.5	0.20	4
			Experiment 26. Normal Ringer				
3:15	1.56	0.56	0.16	0.88	+3.0	0.24	16
3:16	Change to $CaCl_2$-free Ringer						
3:19	1.60		0.16	1.26	−1.0	0.13	9
3:20	Change to normal Ringer						
3:30	1.60	0.56	0.17	0.69	+2.0	0.28	4

TABLE 9

	R-R	P-R	QRS	R-T	AMP. T	DUR. T	R
			Experiment 14. Normal Ringer pH 7.4				
1:52	3.35	0.80	0.17	1.60	+3.0	0.20	11
1:57	Ringer $CaCl_2$ 0.04 per cent 7.4						
1:59	2.72	0.64	0.20	1.54	+3.0	0.28	17
2:02	2.06	0.56	0.16	1.16	−7.0	0.28	10
2:08	1.90	0.52	0.16	1.04	−7.0	0.24	9
2:45	Ringer $CaCl_2$ 0.065 per cent 7.4						
3:10	1.81	0.52	0.16	1.16	−4.5	0.24	14
3:15	1.70	0.52	0.20	0.92	−1.0	0.12	3
3:17	Normal Ringer 7.4						
3:27	2.72	0.80	0.16	1.44	+1.0 −1.0	0.12	6
3:30	3.07	0.80	0.16	1.56	+1.0	0.20	10
			Experiment 25. Normal Ringer pH 7.4				
3:40	1.28	0.44	0.12	0.76	−1.5	0.20	4
3:44	Ringer 0.04 per cent $CaCl_2$ 7.4						
3:53	1.16	0.36	0.18	0.70	−2.0	0.20	4
3:54	Normal Ringer 7.4						
4:00	1.30	0.44	0.12	0.84	−2.0	0.20	4

or more than 7.8. Variations such as Daly and Clark utilized from pH 6.5, ten times less alkaline than the blood, to pH 10.5, a thousand times more alkaline than normal serum, must be regarded as extreme. The

TABLE 10

Experiment 15. Normal Ringer's pH 7.4

	R-R	P-R	QRS	R-T	AMP. T	DUR. T	R
1:48	1.86	0.54	0.12	1.00	+2.5	0.08	14.0
1:53	Change to Ringer 0.06 per cent KCl						
1:54	1.86	0.64	0.12	0.80	−2.0	0.08	11.0
2:00	2.00	Wormlike contractions of ventricle					
2:03	Change to Ringer 0.06 per cent KCl and 0.08 per cent $CaCl_2$						
2:10	1.24	0.65	0.24	0.30	−2.0	0.24	9.0
2:12	1.32	0.70	0.18	0.28	−3.0	0.24	14.0
2:13	Change to Ringer 0.06 per cent KCl						
2:17	Wormlike contraction of ventricle						
2:21	Normal Ringer						
2:25	1.60	0.78	0.14	1.02	−3.0	0.20	14.5
2:45	1.82	0.64	0.14	1.10	−3.0	0.16	9.0

TABLE 11

Experiment 31. Normal Ringer's pH 7.4

	R-R	P-R	QRS	R-T	R	AMP. T	DUR. T	A H M R	V H M R
2:45	1.64	0.50	0.20	1.00	11	−2.0 +1.0	0.20	18	37
2:46	Ringer pH 7.8								
2:51	1.68	0.54	0.16	0.96	8	+4.0	0.20	7	19
2:58	1.56	0.60	0.16	0.90	10	+5.0	0.36	19	20
3:05	1.60	0.70	0.16	0.86	9	+5.0	0.40	25	28
3:12	Ringer pH 7.1								
3:17	1.57	1.00	0.30	1.00	12	+3.0	0.20	23	35
3:27	1.60	0.96	0.28	1.12	14	−1.0 +2.5	0.16	22	43
3:28	Ringer pH 7.4								
3:33	1.66	0.80	0.24	1.08	15	5.0	0.28	16	36

accompanying table illustrates the effects of pH changes in the perfusate.

The results of a number of experiments indicate that the various phases of the electrical activity are affected by changes in pH of the perfusate as follows:

H^+ Increase	*OH^- Increase*
R–R increased	R–R diminished
P–R increased	P–R diminished
QRS increased	QRS diminished
R–T increased	R–T diminished

During such a period of circulatory failure as that described in table 10 the perfusion fluid remains stagnant within the ventricle, the products of tissue metabolism are inadequately removed and the acidity of the perfusate increases. With a view to investigating what effect such a change might exercise upon the action of various concentrations of other kations, Ringer's solutions containing different amounts of NaCl, KCl and $CaCl_2$ at pH 7.8, 7.4 and 7.1 were perfused. From the data thus obtained it appears that the results so produced are the algebraic sum of the effects of the two changes. Thus an increase in acidity and a decrease in $CaCl_2$ both tend to slow the rate of stimulus production. If, now, a heart be perfused with a solution deficient in $CaCl_2$ the effect will be greater if the pH be 7.1 than with a pH of 7.8.

SUMMARY: The following table may be taken as a graphic summary of the effects of the ionic changes as described above.

TABLE 12

		R-R	P-R	QRS	R-T
NaCl	Increase	+	+	−	+
	Decrease	+	+	+	−
KCl	Increase	+	+		−
	Decrease	+	+	+	+
$CaCl_2$	Increase	−	−	+	−
	Decrease	+	+		+
pH	H^+ Increase	+	+	+	+
	OH^- Increase	−	−	−	−

PART II. ARRHYTHMIAS (ABNORMAL MECHANISMS)

Throughout this series of experiments no attempt was made to produce abnormal types of rhythm but when such occurred they were recorded and studied. Subsequently the electrical records were measured with a view to studying the changes in the various phases of the course

of the wave of excitation coincident with the alteration in rhythm. It soon became apparent that there was nothing typical about the arrhythmia produced by a given change in the perfusate.

In the accompanying table the data are arranged as obtained from a study of the records immediately preceding, during, and directly following nineteen examples of abnormal rhythms. In order are given:

1. The change in the perfusate just before the alteration in rhythm.
2. The alterations in the various phases of the electrical record accompanying the occurrence of the arrhythmia.
3. The change in perfusion fluid apparently responsible for the restoration of normal mechanism.
4. The concomitant changes in the electrocardiogram.

In the first seven examples, all of which are types and degrees of block, it will be noted that there is a striking regularity in the changes in the electrical record coincident with the establishment of the new rhythm. In every instance there is a delay in the rate of impulse formation, in auriculo-ventricular conduction, in the rate of spread in the ventricle and in the duration of ventricular systole. The restoration of the normal rhythm, on the other hand, is accompanied by an acceleration in all of these.

We may, therefore, reasonably conclude that the establishment of this abnormal mechanism is secondary to some chemical change which affects the cardiac tissue as a whole..

The remaining twelve instances, however, show no such similarity. There seems to be considerable variation in the electrocardiographic changes accompanying the onset and the offset of the various arrhythmias. Moreover, the same change in the ionic content of the perfusate may be followed by a different type of arrhythmia. Upon analysis it is evident that these examples include various degrees in the establishment of an ectopic pacemaker. It is, furthermore, difficult to escape the conclusion that these abnormal mechanisms are not the result of a generalized chemical alteration in the muscle but of local changes. The effect which the new rhythm, set up at the ectopic focus, may have upon the rhythm of the heart as a whole may be due to diffuse changes in the cardiac tissue, but the essential change giving rise to the aberrant focus in which the new stimulus is produced is more probably local.

BIBLIOGRAPHY

(1) Clark and Daly: Journ. Physiol., 1921, liv, 367.
(2) Lewis: Phil. Trans. Roy. Soc., London, 1916, ccvii, 240.

TABLE 13

ARRHYTHMIA	PRODUCED BY CHANGE FROM: TO:	CHANGES IN E-K				NORMAL RHYTHM PRODUCED BY CHANGE TO:	CHANGES IN E-K			
		R-R	P-R	QRS	R-T		R-R	P-R	QRS	R-T
S-A block	Normal Ringer NaCl 0.75 per cent	+	+	+	+	Normal Ringer pH 7.4	−	−	−	−
A-V block 2:1	Normal Ringer NaCl 0.75 per cent, 7.4	+	+	+	+	Normal Ringer	−	−	−	−
A-V block 2:1, 3:1, 4:1	Normal Ringer NaCl 0.75 per cent, 7.4	+	+	+	+	Normal Ringer	−	−	−	−
A-V block 2:1	Normal Ringer pH 7.4 Normal Ringer pH 7.1	+	+	+	+	Normal Ringer 7.4	−	−	−	−
A-V block 2:1	Normal Ringer 7.4 K-free Ringer 7.8	+	+		+	K-free Ringer 7.1	−	−	+	−
A-V block complete	Normal Ringer K-free Ringer	+	+	+	+	Normal Ringer	−	−	−	−
A-V block complete	Normal Ringer K-free Ringer	+	+	+	+	Normal Ringer	−	−	−	−
Auricular extrasystoles	Normal Ringer Ringer 2.0 per cent NaCl	+	+	−	+	Normal Ringer	+	+	+	−
Auricular extrasystoles	Normal Ringer Ringer 0.2 per cent NaCl	+	−	+	−	Normal Ringer	+	+	−	+

Auricular extrasystoles	Normal Ringer K-free Ringer	+	+	+	+	Normal Ringer	+	+	−	−
Auricular extrasystoles	Normal Ringer K-free Ringer	+	+	+	+	Normal Ringer	+	+	−	−
Ventricular extrasystoles	Normal Ringer Ringer 2.0 per cent NaCl	+	+	−	+	Normal Ringer	+	+	+	−
Ventricular extrasystoles	K-free Ringer pH 7.4 K-free Ringer pH 7.1	+	+	+	+					
Ventricular fibrillation	Normal Ringer Ringer 0.06 per cent KCl	+	+		−	Ringer 0.06 per cent KCl, 0.08 per cent $CaCl_2$	−	−	+	−
Ventricular fibrillation	Ringer 0.06 per cent, KCl 0.08 per cent, $CaCl_2$ Ringer 0.06 per cent KCl	+	+		−	Normal Ringer	+	+	+	+
Ventricular fibrillation	Normal Ringer NaCl 0.7 per cent	+	+	+	+	Normal Ringer	+	+	+	−
Ventricular tachycardia	Normal Ringer pH 7.4 Normal Ringer pH 7.8	−	−	−	−	Normal Ringer pH 7.4	+	+	+	+
Ventricular tachycardia	Normal Ringer pH 7.4 Normal Ringer pH 7.8	−	−	−	−	Normal Ringer pH 7.4	+	+	+	+
Ventricular tachycardia	K-free Ringer pH 7.4 K-free Ringer pH 7.8	−	−	−	+	K-free Ringer pH 7.1	+	+	+	

STUDIES IN THE REGENERATION OF BLOOD[1]

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Studies in the regeneration of blood after simple anemia have been made on many species of animals and on man. Although various phases of the growth of blood have been extensively investigated in connection with destruction of the erythrocytes by chemical agents or specific diseases, much less information is available regarding recovery from the anemias produced by hemorrhage.

Many, if not most, of the earlier researches lack a convincing character because relatively few of the variables have been sufficiently taken into account. Heretofore the work on blood regeneration has been on animals whose dietary control was not complete. Inasmuch as the more recent investigations of nutrition, conducted on laboratory animals, have made it possible to control a variety of dietary factors, only one variable will need to be taken into consideration for the duration of a particular series of experiments.

The present studies on albino rats were undertaken because of the relatively large amount of information available concerning the dietary requirements of these animals. The rat is small enough to make possible the use of large numbers for experimental purposes and it has been employed as a laboratory animal for so many generations that laboratory conditions do not make its confinement unnatural. Our rats were from the colony of the Connecticut Agricultural Experiment Station. They were kept on a standard synthetic diet[2] while in experi-

[1] Material for this paper was taken from the dissertation submitted for the degree of Doctor of Philosophy, Yale University, 1921.

[2] Standard diet used:

	per cent
Cornstarch	50.5
Casein	18.0
Lard	18.0
Washed butter	9.0
Salt mixture	4.5

mental use and during all of the time when they were not under direct observation. This eliminated complications arising from having the animals eat such complex mixtures as they would, had they been fed table scraps.

Technique. The preliminary experiments were designed to discover the limit of successful bleedings in the rat, the period for regeneration of blood after hemorrhages, and to find the effect of the dietary factors on such regeneration.

The rats on which hemorrhages were produced were kept in iron-wire cages with paraffined tin cups for water and porcelain cups for food. The methods and technique employed have been described by Miss Ferry (1). The standard synthetic diet developed by the work of Osborne and Mendel (2), (3), (4) and others was used as a basis for all the dietaries employed. Adult and young animals of both sexes were used, varying in average weight from 100 grams for the young to 300 grams for the adults. The tables of Donaldson (5) were carefully followed in estimating the blood volume of each experimental animal used.

Experimental technique. The blood of every rat was carefully examined for the number of erythrocytes and percentage of hemoglobin before any hemorrhages were made. The counts were checked in every case during the first 2 months until the operator had attained sufficient skill to make such duplications unnecessary. The method of blood count was the familiar one with a Thoma-Zeiss hemocytometer and the use of either Hayem's or Toisson's solution for blood dilution. Hemoglobin was determined by the colorimetric procedure of Cohen and Smith (6). Rat blood was used as a standard and its color was checked by fresh material at frequent intervals.

The sample of blood for examination was taken from a drop extruded when the end of the tail was snipped. The rat was placed in an open glass bottle, the bottom of which had been removed and a tin cap with a hole for the tail placed over the opening; leather straps from the neck of the bottle held the rat securely in this armor (see fig. 1). This arrangement allowed free manipulation of the tail by the experimenter. The skin of the tail was softened with mineral oil. Previous to removing the end of the rat's tail it was placed in warm water or brushed over with toluene in order to suffuse the blood vessels. There are four blood vessels in the rat's tail, one dorsal, one ventral and two lateral. The blood was obtained from these by manipulating the tail after the fashion of milking and allowing the fluid thus "milked" out to drop into a

tared glass dish. At the beginning of this series of experiments, hemorrhages of from one-third to one-half of the estimated total blood volume were produced. After "milking out" 20 or 30 drops a clot usually formed; this was carefully wiped off with a previously weighed piece of cotton and added to the blood in the dish. Then more blood was worked out of the end of the tail. The blood and vessel were weighed rapidly to centigrams—two or three times, if necessary—until at least one-third of the whole blood volume contained in the rat had been removed. This method which was laborious and unsatisfactory resulted in occasional injury to the tail accompanied in some cases by

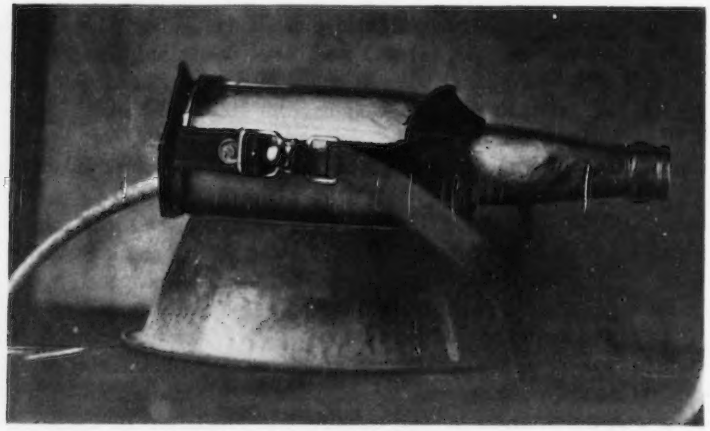

Fig. 1. This shows a rat held in the bottle so that the tail is exposed for "milking" out blood.

infection. Frequently a second hemorrhage was desired before the wound on the tail was completely healed. Because of this soreness and the possibility of infection it often became impossible to obtain a sample of healthy blood for cell count and hemoglobin determination.

Subsequently, therefore, the method used for obtaining blood from guinea pigs in serum work was adopted.[3] In this procedure blood is secured directly from the jugular vein. The rat was anesthetized with ether, the fur closely clipped from its neck, a slit about ½ inch long made in the skin, the jugular exposed, a v-shaped nick made in the vein with

[3] Thanks are due my co-worker, Dr. W. G. Karr, for his kindly suggestion as to use of this method and for his assistance in the first operations.

scissors, and the animal held over a weighed glass vessel into which the blood was allowed to drop. If care was exercised it trickled slowly and in a well-directed stream and scarcely touched the fur. The blood was allowed to flow into the dish up to the mark previously placed to indicate one-third the estimated total volume of blood. By the time this had run out a clot had usually formed. The clot was carefully and gently wiped off from the wound with a previously weighed bit of cotton, and this added to the blood in the dish, and the whole was then weighed. If a clot failed to form the bleeding was stopped by pressing a small (weighed) piece of cotton against the cut in the vein. Ordinarily this proved effective. If less than one-third of the (estimated) rat's blood had run out by the time a clot had formed, it was easy to start the bleeding again by harshly wiping the nick in the vein with a weighed pledget of cotton; then more blood dripped and a second weighing was made. This second bleeding was, however, seldom necessary. The wound in the skin was then washed with dilute alcohol (25 per cent) and sewed up. The rats recovered readily, the wound healing in from 2 to 3 days in every case.

Blood counts and hemoglobin determinations were made daily until the original values returned. During the period of regeneration fragmentary red cells were often seen in the blood, but as regeneration proceeded these became fewer in number and finally disappeared altogether. The number of leucocytes increased the first day or two after the hemorrhage, but not in great abundance. As shown by Lamson (7) the number of erythrocytes as well as the percentage of hemoglobin varies among the individuals, but the drop after hemorrhage in each case is comparable.

Criteria of regeneration. The return of the number of red blood corpuscles and the percentage of hemoglobin to the values noted before any loss of blood had been sustained by the rat were the criteria used in judging regeneration. These values remained approximately constant for two successive days before the rat was considered to have regenerated his blood. For normal animals of either sex fed on standard diet (see footnote 2) with the addition of dried brewery yeast, the time was from 6 to 9 days (table 1). This has been corroborated by the subsequent studies of Geiling and Green (8) in this laboratory. At the end of the period required for regeneration, in many cases a second hemorrhage required about the same time for a return to normal blood conditions. A third hemorrhage was made on some of these rats, but regeneration in such animals always needed more time. If, however,

a week's time intervened between return to normal and the following bleeding, the regeneration rate was normal. Similar experiences have been recorded by Geiling and Green for double hemorrhages.

Blood regeneration in growing rats. To study the effect of loss of blood on the growth of young animals bleedings were made on healthy growing rats fed on the standard diet and brewery yeast as the adults were fed, the hemorrhage procedure being the same as for the grown rats. The time of total regeneration for these little rats was from 7 to 9 days (see table 2). After the hemorrhage they recovered quickly,

TABLE 1

Blood regeneration in adult rats after a single hemorrhage on the standard diet

BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPUSCLES*	HEMOGLOBIN	TIME FOR REGENERATION
grams	*grams*	*grams*	*per cmm.*		*days*
220	130	5.5	9,360,000	91	
			10,136,000	104	7
210	12.5	5.5	9,472,000	100	
			9,168,000	87	9
272	15.7	5.5	8,256,000	94	
			9,008,000	87	8
310	17.5	7.0	9,264,000	104	
			9,504,000	91	6
250	14.5	5.1	9,500,000	110	
			9,104,000	98	7
210	12.4	5.0	9,000,000	94	
			9,100,000	96	7
300	17.4	5.0	9,664,000	107	
			8,800,000	91	8

* The figures in the tables under the heading Red Blood Corpuscles give the number of erythrocytes before hemorrhage and the number at the end of recovery.

continued to grow and increase their body weight in a normal manner. Apparently the loss of one-third of their blood did not hinder growth in any observable respect. Some young animals whose growth had previously been delayed by lack of vitamin B in experiments by Cajori (9) were also used, but these recovered in the same way as the animals showing uninhibited growth.

Diet and dietary factors. In order to make these experiments as accurate as possible and to eliminate some of the variables occurring in other work on blood regeneration, the standard diet, with the addition of dried brewery yeast, was used. Diets of each component of this

composite food were used, that is, protein only (casein); carbohydrate only (cornstarch); fat only (washed butter); protein-free food (butter, lard, cornstarch and salt mixture)[4] and one food accessory (vitamin B) in a relatively concentrated form. The sources of the food accessory

TABLE 2

Blood regeneration in growing rats after a single hemorrhage on standard diet

BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPUSCLES	HEMOGLOBIN	TIME FOR REGENERATION
grams	*grams*	*grams*	*per cmm.*		*days*
92	6.1	2.1	11,264,000	100	
			8,500,000	100	9
95	6.3	2.1	9,536,000	100	
			7,752,000	96	9
85	6.0	2.25	10,880,000	110	
			9,312,000	110	9
111	7.2	2.4	8,256,000	94	
			7,442,000	96	7
112	7.2	2.4	10,336,000	114	
			9,440,000	98	7
103	6.77	3.0	9,504,000	110	
			8,448,000	94	7
113	7.2	2.5	10,000,000	110	
			9,109,000	110	7

TABLE 3

Blood regeneration in adult rats after a single hemorrhage on a mixed diet

DIET	BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPULCLES	HEMOGLOBIN	TIME FOR REGENERATION
	grams	*grams*	*grams*	*per cmm.*		*days*
Dog biscuit.....	322	18.34	8.2	10,192,000	94	
				8,400,000	98	7
	320	18.0	6.3	10,080,000	100	
				8,912,000	98	7
Table scraps.....	310	17.7	5.25	8,812,000	78	
				8,800,000	96	10

were dried brewery yeast, yeast concentrate[5] (10), tomato juice and orange juice.

[4] Salt mixture, Osborne and Mendel, Journ. Biol. Chem., 1919, xxxvii, 572.

[5] Yeast concentrate, Osborne and Wakeman (10).

Mixed diets. In order to learn what difference, if any, existed between the effect of our standard (synthetic) diet and that of scrap food on the rate of blood regeneration, two rats were fed on dog biscuit for 2 days before bleeding and then during the entire period of the manufacture of new blood. The results are shown in table 3. In both cases the organism regenerated hemoglobin more rapidly than red cells. This may have been merely accidental, but the same circumstances occurred in the case of one rat fed on table scraps (meat, lettuce, bread, etc.). This opens the question as to what factor or factors are here present which we did not have in the standard basal diet with added yeast as a source of vitamin B.

TABLE 4

Blood regeneration in adult rats after a single hemorrhage on a limited food intake—5 grams per day

BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPUSCLES	HEMOGLOBIN	TIME FOR REGENERATION
grams	*grams*	*grams*	*per cmm.*		*days*
292	16.7	5.8	10,000,000	91	
			9,128,000	90	7
320	18.3	6.4	8,256,000	94	
			8,480,000	90	7
240	14.0	4.8	9,360,000	91	
			8,886,000	91	7
230	13.4	4.6	9,472,000	100	
			9,776,000	104	6
200	11.9	4.0	7,328,000	70	
			8,128,000	72	6

Underfeeding. A series of hemorrhages was made on animals with a limited supply of food, i.e., 5 grams per animal per day, including 0.2 grams yeast daily added to the standard basal diet as above. This limited food consumption did not prolong the duration of regeneration appreciably, the normal being reached from 6 to 7 days after the initial loss of blood (table 4). Loss of body weight occurred in each case, as might be expected since the caloric intake was insufficient, but the animals remained healthy and active throughout the period of limited feeding.

Starvation. When animals were not allowed any food throughout the period of blood regeneration, the rate of return to normal hemoglobin value and number of erythrocytes was slower than in the above cases, the period for one animal being as long as 13 days. A control rat with-

out hemorrhage was starved at the same time; its blood was found to vary no more in red cell count and hemoglobin percentage from day to day than that of a normal animal eating as much as it desired. Normal rats do not maintain wholly constant values for either hemoglobin percentage or number of red cells. This slower rate of regeneration during inanition is in agreement with the results of Hooper and Whipple (11) on fasting dogs after simple anemia (table 5).

Protein only. Since casein is the protein used in our standard synthetic diet and has been shown to be an adequate protein (2), this was fed as a sole source of nourishment to another group after hemorrhage until blood regeneration was accomplished. Only 2 grams per day per animal were used after it was found that the rats refused to eat more than this amount in that period. This limited protein diet was used to see whether in accord with the experience gained with the protein-

TABLE 5

Blood regeneration in adult rats after a single hemorrhage; during this period they received no food

BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPUSCLES	HEMOGLOBIN	TIME FOR REGENERATION
grams	*grams*	*grams*	*per cmm.*		*days*
340	19.2	7.5	8,860,000	114	
			8,900,000	110	13
330	18.7	6.6	8,562,000	74	
			9,728,000	87	10

rich diet of dogs (11) the rate of regeneration was more rapid than on other diets of one food factor only.

The time of blood regeneration in this set of experiments was from 9 to 12 days, more rapid than in starvation but not so rapid as on some other equally limited diets (table 6). It must be borne in mind however that no food accessories (vitamins A and B) were present and hence the animals ate less than they otherwise would. The energy intake was obviously inadequate so that underfeeding complicated the problem. In all these feeding periods where the diets were limited in amount or kind, or both, the rats were more coprophagous than normal, no doubt in an effort to satisfy their desire for a feeling of fullness.

Fat only. In feeding the fat portion of the basal diet, butter washed free of salt was used in an amount corresponding to the daily weight of protein fed. The rats did not eat the butter readily and never ap-

peared so anxious for their food as did the animals on the other diets. The time required for regeneration of blood by this set of fat-fed animals was 9 days, not longer than for the protein-fed rats (table 7).

Carbohydrate only. Two grams of cornstarch, made in the form of a paste with water to prevent scattering, were fed daily to each of three rats after they had been bled. These animals required 12 days for

TABLE 6

Blood regeneration in adult rats after a single hemorrhage on a protein diet—2 grams casein per day

BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPUSCLES	HEMOGLOBIN	TIME FOR REGENERATION
grams	*grams*	*grams*	*per cmm.*		*days*
290	16.7	7.7	10,656,000	96	
			10,032,000	96	9
180	11.9	4.25	11,072,000	104	
			10,500,000	105	12
153	10.2	3.9	10,432,000	100	
			9,600,000	100	12
158	10.56	3.0	10,336,000	104	
			10,112,000	104	9

TABLE 7

Blood regeneration in adult rats after a single hemorrhage; on a diet of butter fat only —2 grams per day

BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPUSCLES	HEMOGLOBIN	TIME FOR REGENERATION
grams	*grams*	*grams*	*per cmm.*		*days*
295	17.0	7.0	8,528,000	98	
			9,136,000	98	9
355	20.0	9.0	8,480,000	76	
			7,800,000	80	9
165	11.0	4.0	10,416,000	91	
			9,440,000	80	9

complete regeneration. This is somewhat longer than for those on the protein diet, in agreement with the work on dogs receiving a carbohydrate-rich diet (11) (table 8).

Protein-free food. This food was made up after the formula for the standard diet with the casein replaced by corn starch, making a food containing 68.5 per cent corn starch, 18 per cent lard, 9.0 per cent butter and 4.5 per cent salts. Three animals were given 5 grams of

this protein-free food daily, hemorrhages were made and the process of blood regeneration watched by the methods used before. These rats replenished their blood supply in from 9 to 12 days (table 9), slightly more time being required in this series than in the case of the diets of fat only, but in one case less than with carbohydrate only. Yeast was fed with this protein-free food to the extent of 0.1 gram per day but the exceedingly small amount of protein therein evidently had little

TABLE 8

Blood regeneration in adult rats after a single hemorrhage; on a carbohydrate diet—2 grams cornstarch per day

BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPUSCLES	HEMOGLOBIN	TIME FOR REGENERATION
grams	*grams*	*grams*	*per cmm.*		*days*
150	9.5	3.2	10,288,000	90	
			9,568,000	91	12
395	22.0	8.5	11,600,000	116	
			10,368,000	114	12
240	14.0	6.0	9,600,000	91	
			9,696,000	94	12

TABLE 9

Blood regeneration in adult rats after a single hemorrhage; on a protein-free diet—5 grams per day

BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPUSCLES	HEMOGLOBIN	TIME FOR REGENERATION
grams	*grams*	*grams*	*per cmm.*		*days*
195	11.6	4.47	8,976,000	87	
			8,496,000	90	9
255	14.8	6.0	10,896,000	110	
			10,392,000	107	12
190	11.45	5.3	10,000,000	110	
			10,400,000	110	12

or no effect on the speed of blood-cell formation. In all cases it was impossible to keep the animals eating this ration without the addition of the dietary essential, vitamin B. This led to work on diets without added yeast, with yeast as a sole source of nourishment, and with other vitamin-containing products, such as tomato juice and orange juice alone.

Standard diet without yeast. Four rats were supplied with an unrestricted quantity of standard diet and an abundance of fresh water, but

no yeast, i.e., no vitamin B, was given to them; they were bled according to the regular procedure and their recovery was apparently normal. They ate less and less as time went on, as has been the case for all animals with lack of vitamin B (12), and they lost in weight. The return to normal blood values came in from 12 to 13 days; in three of the four cases hemoglobin values were low. This could not be due to lack of iron, for some iron was eaten in the salt mixture, although more iron would have been consumed had yeast also been added to the diet (table 10).

Yeast only. Because of these results with protein-free food and with other diets without yeast, a series of hemorrhages was made on rats which were fed nothing but yeast during the period of recovery. Ac-

TABLE 10

Blood regeneration in adult rats after a single hemorrhage; on the standard diet, without yeast

BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPUSCLES	HEMOGLOBIN	TIME FOR REGENERATION
grams	*grams*	*grams*	*per cmm.*		*days*
295	170	6.48	11,648,000	107	
			11,292,000	96	13
315	180	6.0	10,112,000	91	
			10,288,000	94	12
355	200	6.9	11,584,000	114	
			12,100,000	104	12
270	156	5.4	10,768,000	98	(Sick)
			10,068,000	85	13

cordingly four animals were selected which had responded in a normal manner to regeneration after experimental anemia several weeks previous to this time and each was again subjected to a hemorrhage. These rats were then fed 0.5 gram dried brewery yeast daily. They ate the yeast with great avidity as soon as it was offered. This amounted to practical starvation, and although they lost body weight the animals all regenerated their blood in from 6 to 8 days (table 11). In each case the hemoglobin values were equal to or exceeded the original. This group of animals regenerated their blood in less time than the group without food.

As a check for these results with yeast alone a group was given 0.5 gram protein (casein) daily to see if the small quantity of yeast protein was the stimulant to forming new blood or whether it was in truth the

food accessory in the yeast. The time required for these three animals to regenerate their blood was 7 to 10 days. The hemoglobin values were again low in two cases as for the animals which were fed no yeast (table 12). The casein contained no iron but the rats were in iron-wire cages and continually gnawed at the wires.

TABLE 11

Blood regeneration in adult rats after a single hemorrhage; on a diet of yeast only—0.5 gram per day

BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPUSCLES	HEMOGLOBIN	TIME FOR REGENERATION
grams	*grams*	*grams*	*per cent*		*days*
292	16.76	6.5	11,184,000	112	
			10,416,000	112	8
320	18.0	6.2	9,134,000	90	
			9,648,000	98	7
290	16.7	7.0	10,784,000	96	
			10,336,000	98	6
355	20.0	7.0	10,144,000	102	
			10,656,000	104	7

TABLE 12

Blood regeneration in adult rats after a single hemorrhage; on a protein diet—0.5 gram casein per day

BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPUSCLES	HEMOGLOBIN	TIME FOR REGENERATION
grams	*grams*	*grams*	*per cmm.*		*days*
147	9.34	3.2	8,704,000	102	
			9,036,000	98	7
150	9.5	3.0	12,000,000	108	
			9,924,000*	104	7
157	10.5	4.1	11,040,000	87	
			11,770,000	91	10

* R.B.C. constant 3 days.

Water soluble vitamin (B). Two products other than yeast, rich in this dietary essential, were used as a sole nutrient for a group of rats which had experienced hemorrhages. Orange juice, containing no iron (by analysis and according to Sherman (13)) and tomato juice containing no iron (by analysis and according to Sherman) were fed to two sets of animals. The orange juice was prepared by straining a

mass of crushed pulp and juice through four layers of clean cheesecloth. This resulted in a perfectly clear fluid which was measured out for feeding by means of a pipette. The tomato juice was the juice from canned tomatoes, from which the pulp and seeds were separated by

TABLE 13

Blood regeneration in adult rats after a single hemorrhage; on a diet of orange juice only—vitamin B—5 cc. per day

BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPUSCLES	HEMOGLOBIN	TIME FOR REGENERATION
grams	*grams*	*grams*	*per cmm.*		*days*
250	13.5	4.9	12,384,000	114	
			11,548,000	114	6
118	7.7	2.8	12,000,000	110	
			11,880,000	112	6
170	11.2	3.2	9,776,000	102	
			10,608,000	112	6
275	16.	5.3	10,528,000	107	
			10,608,000	102	6

TABLE 14

Blood regeneration in adult rats after a single hemorrhage; on a diet of tomato juice only—5 cc. per day

BODY WEIGHT	TOTAL BLOOD	AMOUNT OF HEMORRHAGE	RED BLOOD CORPUSCLES	HEMOGLOBIN	TIME FOR REGENERATION
grams	*grams*	*grams*	*per cmm.*		*days*
400	22.3	5.5	10,768,000	104	
			11,682,000	107	6
225	13.0	4.7	10,192,000	100	
			10,176,000	100	6
367	20.5	8.7	10,808,000	100	
			10,880,000	96	8
305	17.5	5.6	8,592,000	94	
			8,352,000	92	6

straining through four layers of cheesecloth. This was also measured with a pipette. The four rats on 10 cc. of orange juice daily, reached their normal blood values in 6 days (table 13), those on the tomato juice, 5 cc. daily, (they refused to eat as much as 10 cc. per day) recovered in from 6 to 8 days; again the hemoglobin values varied (table 14).

SUMMARY OF RESULTS

Hemorrhages of approximately one-third of the estimated total blood volume can be borne by albino rats without interfering with complete recovery. When one-half or more of the blood was removed by hemorrhage, death ensued. The criteria used in judging blood regeneration in the rat were the return of the number of erythrocytes and the percentage of hemoglobin to the values noted before the animal had sustained any loss of blood.

By varying the diet from elimination of one factor to elimination of all but one factor therein, the speed of regeneration of blood was changed, but with each diet the organism replenished its normal supply of blood to the original values for red cells and hemoglobin.

Protein permitted more rapid blood regeneration than either carbohydrate or fat when fed as a sole nutrient (2.0 grams daily).

The diets of vitamin-rich food, even in very small amounts, gave somewhat more speedy regeneration than any other diet containing one food factor only.

This study of blood regeneration has not covered the effect of iron on blood formation. Work on this phase of the problem is now being carried out.

I desire to express my sincere thanks to Prof. Lafayette B. Mendel who outlined this problem, for his suggestions and interest throughout the investigation.

BIBLIOGRAPHY

(1) Ferry: Journ. Lab. Clin. Med., 1920, v, 735.
(2) Osborne and Mendel: Journ. Biol. Chem., 1912, xiii, 233.
(3) Osborne and Mendel: Journ. Biol. Chem., 1913, xv, 311.
(4) Osborne and Mendel: Journ. Biol. Chem., 1915, xx, 351.
(5) Donaldson: The rat, Philadelphia, 1915.
(6) Cohen and Smith: Journ. Biol. Chem., 1919, xxxix, 489.
(7) Lamson: Journ. Pharm. Exper. Therap., 1915, vii, 169.
(8) Geiling and Green: Proc. Soc. Exper. Biol. and Med., 1912, xviii, 191.
(9) Cajori: Journ. Biol. Chem., 1920, xliii, 583.
(10) Osborne and Wakeman: Journ. Biol. Chem., 1919, xl, 383.
(11) Hooper and Whipple: This Journal, 1918, xlv, 573.
(12) Karr: Proc. Soc. Exper. Biol. and Med., 1920, xvii, 84.
(13) Sherman: Chemistry of food and nutrition, New York, 1919, 2nd ed., 335.

THE HEAT LIBERATED BY THE BEATING HEART

Third Communication

The Oscillations of Temperature During the Cardiac Cycle, or the Thermocardiogram of the Terrapin

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The problem. In former papers (1), (2) it has been shown that the temperature of the terrapin's surviving ventricle rises and falls with each beat with a regularity and apparent smoothness equal to the mechanical changes of the beat. The observations were made in the one case by use of the Paschen, in the other by use of the Einthoven galvanometer. Since then the same thing has been observed by using a sensitive form of a d'Arsonval galvanometer. In this last case records were at first gotten like the one shown in figure 1. Here it will be seen in a very graphic way that the temperature begins to rise with systole and continues so to rise apparently until late diastole. The cooling also appears to take place all at one time to be interrupted only at the beginning of the next systole. The curve obtained, in short, is very like those obtained in the previous experiments, but on account of the different method used, more sharply marked.

The question now arose, would the temperature graph continue to be so simply diphasic if the heart were made to beat more slowly, thus giving the galvanometer more time to follow the detail of temperature oscillation? For in the interpretation of the responses of a galvanometer to changing differences of potential one must allow for the period of the instrument. In the graph of figure 1 the duration of the heart beat was about 15 seconds, but the period of the galvanometer was 16 seconds; that is, 32 seconds were required for it to respond to a given current and then return again to null point. Obviously the instrument could not follow detailed oscillations of temperature, and the question whether the rise occurs in intervals separated by falls could not have been determined in the experiment of which figure 1 is a record.

One thing however appears directly in the graphs of this, that also appeared in the former experiments. The cooling during a cardiac cycle offsets the warming, so that if one compares a long series of beats one sees no evidence that the organ at the end of the series is a warmer body than it was at the beginning. The general temperature of the heart apparently has reached a point where the rate of heat loss by conduction and radiation just equals the rate at which the heat at each beat is produced.

Nevertheless to know more about the details of temperature changes during the single cardiac cycle it is obvious that one must select either

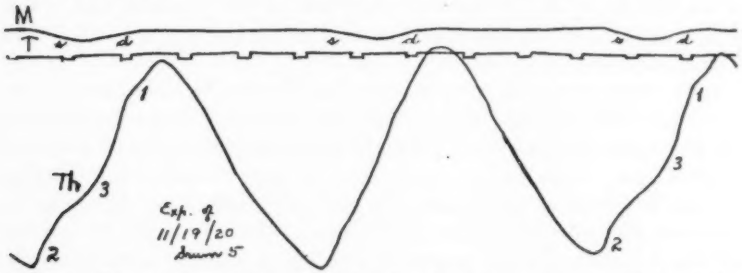


Fig. 1. Line tracing of drum record reduced to eleven twenty-eighths of the original size. In this as in all the subsequent records read from left to right. The upstroke of the thermocardiogram, *Th*, indicates rise of temperature, the down stroke fall of temperature of the heart muscle. The down stroke of the cardiomyogram, *M*, indicates the systolic phase of the heart beat, the upstroke indicates diastolic phase: these are marked *s* and *d* respectively. The time trace, *T*, always marks intervals of three seconds each. In later figures along the vertical, *S*, are the "scratch" marks indicating the relative positions of the lever writing tips with drum at standstill. In the present record, not shown in the figure, the scratch marks indicate that all the writing tips lie on the same vertical.

a galvanometer of shorter period or a heart beating at a slower rate. In the present paper I propose to give the results of choosing the latter alternative.

METHOD AND INSTRUMENTARIUM. As in my former investigations the thermoelectric method of measuring temperature was employed. The insert form of thermopile of 30 pairs of iron-constantan junctions again was used. The galvanometer, as stated, was a special form of the d'Arsonval type, having a sensitivity of about 23 mm. of scale at distance of 1 M to a difference of 1 microvolt potential. The internal resistance of the galvanometer was 12 ohms, its critical (external) damping resistance 30 ohms, the period was 16 seconds.

The heart chambers used are made of "vacuum food jars"; a larger type provided with a thick hard rubber cover, a smaller type with an india rubber (stopper) cover. From the inner surface of the cover are suspended the necessary rods (of hard rubber) providing first a fixed point for attaching the base of the heart, second leads for the wires connecting the thermopile terminals with their external binding posts. A median perforation, 0.2 mm. in diameter provides passage way for a suspension thread from the apical tendon of the ventricle to the outside of the jar and thence to a muscle lever held in a fixed position above the jar. The lever is a carefully calibrated isometric form with a Ludwig vertical writing style attached at its recording end. The mechanical action of the ventricle, or the cardiomyogram, may thus be registered upon a revolving drum.

The temperature changes of the ventricle, or the *thermocardiogram*, as indicated by the movements of the galvanometer spot, were recorded on the drum synchronously with the myogram by means of a special contrivance. This latter consists of a short needle-like indicator mounted erect on a carriage that in turn rests upon a rail set along the lower margin of the galvanometer scale. A cable attached to one end of the indicator carriage passes over a pulley wheel at one end of the scale thence drops down vertically to its final point of fixation, namely, to one end of a horizontal writing style, the writing style being held free from lateral vibrations by vertical guide bars. The indicator can thus be set at the position of the galvanometer spot on scale and made to follow the deflections of the galvanometer by the observer. In doing this the writing style is made to move vertically the same distance as the scale indicator is made to move horizontally. The drum tracing of the galvanometer deflections as well as the movements of the myograph with the drum at standstill describe vertical lines, and no correction for arc is involved in reading the records. An instrument similar to this in idea, although for quite a different purpose, is described by Spaeth (3).

The *time marker* and *signal-key* is a specially combined electromagnetic chronograph and electric signal-key. The writing style, while recording whatever time interval is chosen, also by means of an extension of the receiving end of the lever and by properly placed contacts and insulation, records, for instance, the moment of sending a calibrating current through the galvanometer. The drum record thus may bear in addition to the heart tracings a permanent record of the galvanometer performance at the time of the experiment.

Since the *sine qua non* of the experiments is a slower rate of heart beat, the method of the cooled heart chamber and the split ventricle, already described in my paper of 1917, has been of especial value (1). By cooling alone one could easily have a rate of one beat per minute. By the blocking sometimes accomplished in splitting the ventricle, the rate of beat may be reduced to one a minute at higher temperatures,—a very important deviation of the method when one wishes to separate the beats with a greater time interval without reducing the metabolism rate involved in the single beat.

The method of the split ventricle has another importance equal to if not greater than that of slowing the beat. It enables one to use the flat-coil or "Gittersäule" form of thermopile (4). I have called it the insert thermopile, the thermopile being inserted between the halves of the ventricle. This is by all odds the best form for applying the warm junctions to the tissue. In combination with the isometric lever the chances of the junctions slipping or pulling loose during the muscle's activity are reduced to a minimum.

The cold junctions in these experiments were sometimes covered with filter paper wet with water or serum, sometimes with strips of pericardium or pectoralis muscle fresh from the animal that furnished the heart.

The records often contain "scratch marks" showing the position of zero as well as initial tensions of the muscle lever. From the chart of values in terms of grams tension previously determined for the muscle lever, its absolute tension-values can thus be calculated and assigned to the various myograms of an experiment.

The absolute values of the thermocardiograms in these experiments can only be calculated from the theoretical performance of the thermopile as it was used, and the sensitivity of the galvanometer. Since this latter factor varied somewhat during the course of the investigation, the thermal values, for the sake of more accurate comparison, were reckoned out on this basis. The impossibility of using the direct method of calibrating a thermopile and galvanometer with any but parallel fibered muscle has been pointed out by Hill (5). Furthermore the error from the indirect method of calibrating the apparatus according to Bürker (6) is quite negligible. Since with a terrapin's ventricle whose cross section is not constant Hill's method was clearly out of the question, there was no alternative in the present case.

To sum up, the set-up of apparatus for the present experiments enables one to have on drum records the following data:

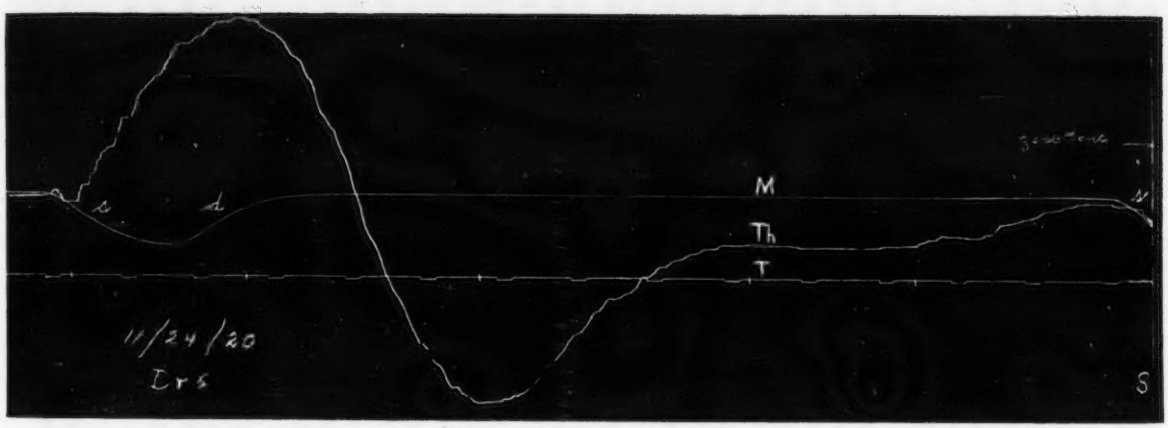


Fig. 2. Original drum record reduced to seventeen-thirtieths of the original size, from experiment of 11/24/20 drum 6. For interpretation of tracings see legend to figure 1. The tensions exerted during the beat were initial tension 22.8 grams, maximal tension during systole 44.8 grams. The zero and initial tensions are indicated by scratch marks at right margin.

1. The performance of the isometric lever—zero tension, initial tension, and

2. The various tensions developed during the heart's activity over a period of time, or the cardiomyograms.

3. The performance of the galvanometer,—the steadiness of the null point over a period of time and the frequency of serious external disturbances, the promptness of response and the distance of deflection to a current of known strength (the sensitivity), the period of time required for this deflection, the amount of dead beat or aperiodicity prevailing, and

4. Sometimes the prevailing difference of temperature between the cold and warm junctions of the thermopile a half-hour after it has been placed in the heart is so small that the swing of the galvanometer from null, when the thermopile is thrown in circuit, can also be registered on the drum record. Finally

5. One is able to register synchronously with the myocardiogram the accompanying temperature changes of the ventricle during the transitions from systole to diastole, through the pause, and back again to systole,—in short, the thermocardiogram.

Description and critical analysis of results. As already indicated the results here presented have their chief value in the fact that the hearts were made to beat slowly. This condition made it possible to use a convenient galvanometer of high microvolt sensitivity, an instrument that in spite of its long period could follow in greater detail the oscillations of temperature occurring in the organ during each beat. What is meant by detail of temperature oscillation is strikingly shown by a comparison of the records of two different experiments as illustrated in figures 1 and 2. In the first the duration of the heart beat is about 15 seconds. In the second it is about 42 seconds. In the first the temperature change seems to be made up of a simple rise with each beat followed by a simple fall, this fall to be interrupted in turn by the rise occurring with the next systole. In the second experiment (fig. 2) each heart beat clearly entails three distinct rises of temperature, the first two of which are separated from each other by a distinct fall, a fall equal to the sum of the first two rises, while the second and third periods of temperature rise are separated by a temperature stasis.

In figure 3 we have the record of another heart the duration of whose beat is about 33 seconds. The thermocardiogram in this case is made up of two distinct rises of temperature each followed by a fall, the

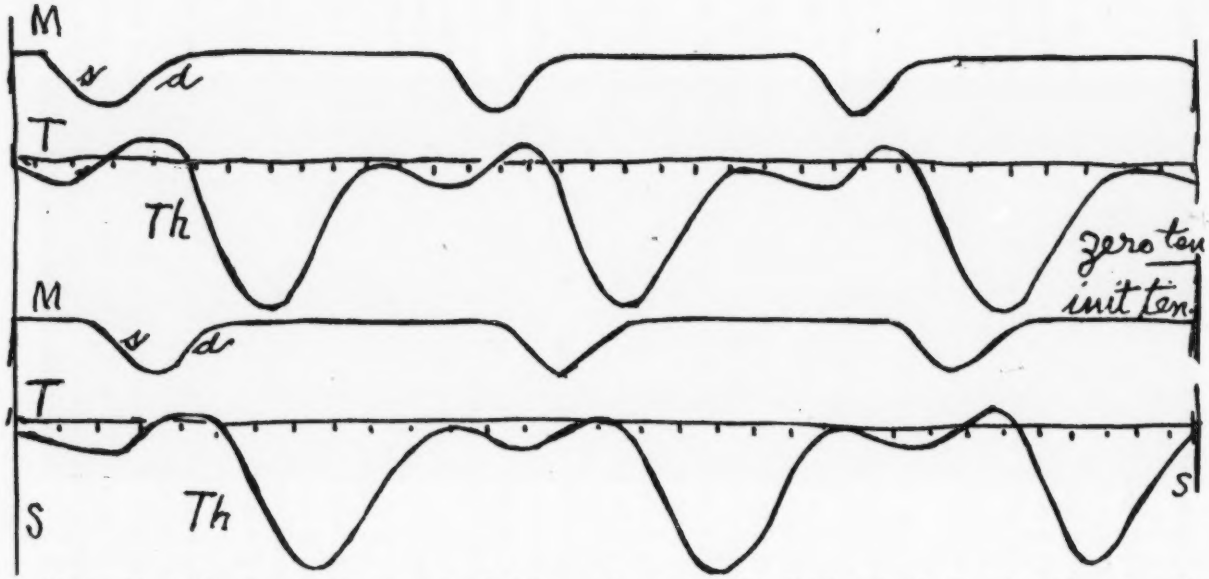


Fig. 3. Line tracing of a part of drum record no. 2 of the experiment of 2/26/21, slightly reduced. Duration of beat about 33 seconds. The second and third rises of the polyphasic graph are here fused into one. For additional experimental data and analysis see table 1 and page 267. For explanation of lettering see legend for figure 1.

first of which is as great as the sum of both rises. Figure 4 (May 25) exhibits another heart whose beats cover periods more than 120 seconds, and here again the rise of temperature occurs at separate intervals, but in three very distinct intervals instead of two, as in the preceding case. A distinct fall of temperature again appears between the first two rises.

If one compares the durations of cardiac cycles with the number of temperature phases appearing in their respective thermocardiograms, it becomes evident that the period of the galvanometer is the determining factor, within certain limits, of the phase number, as appears in the following:

DATE OF EXPERIMENT	DURATION OF CARDIAC CYCLE IN SECONDS	NUMBER OF TEMPERATURE PHASES* PER CYCLE
11/19/20 (See fig. 1)	14	2
5/ 4/21 (See fig. 5)	24	2
2/26/21 (See fig. 3)	33	4
11/24/20 (See fig. 2)	42	5
5/ 4/21 (See fig. 6)	51 to 61	5
6/ 4/21 (Drum. 3)	87	5 or 6
5/25/21 (See fig. 4)	170 to 230	5 and 6

* The term, temperature phase, is applied to any well-marked temperature stasis as well as to a well-marked rise or fall of temperature occurring during the cardiac cycle.

This relation between galvanometer period and duration of cardiac cycle in effecting a change from a diphasic to a poliphasic thermocardiogram is most convincing when one finds records of it in one and the same heart, as has occurred in several of my experiments as the heart rate increased in duration say from 24 to 50 or 60 seconds. (See the table above, exper. of 5/4/21.)

To what extent all the galvanometer deflections in a polyphase thermocardiogram represent actual temperature changes in the ventricular tissue may now be given consideration. At the outset it may be stated that periodicity is what one would expect when recording a galvanometer's deflections. At any rate, wherever a number of oscillations growing successively smaller follow on the heels of an initial deflection one may well suspect that one's instrument is not dead-beat. But control drum records of the performance of my instrument from time to time show that it was beautifully dead-beat up to about June 5, 1921, when some excessive twist permanently changed the flat-strip suspension filament. Records from this date on show a tendency

toward periodicity in the response to the calibrating current, and the thermocardiograms differ correspondingly. Those for the longest cardiac cycle (160 seconds, exper. of 6/5/21) show as many as 7 phases, and negative phases are larger or replace phases of temperature stasis.

Careful examination of records taken when the galvanometer was known to be dead-beat shows that negative (cooling) phases may appear after the second positive phase. These later oscillations therefore can not be attributed altogether to periodicity of the galvanometer.

Indeed careful examination of all my records has convinced me that a terrapin's normal thermocardiogram is made up of five or six phases, whose character is similar to those shown in figure 2 for the faster, and in figure 4 for the slower heart beats. I shall now proceed to an analysis of these various phases as best I may from the data of my experiments.

1. The first positive oscillation or *the first temperature rise, phase 1.* This phase begins with, or just after, the first rise of tension in the heart muscle as shown in the cardiomyogram (down stroke in the records). The muscle lever is always set with considerable initial tension on the muscle, 14 grams and more. The beginning of systole, as the records show it, therefore does not mark the earliest rise of tension but only the moment when it exceeds the initial tension. But even so, this first rise of temperature usually appears a little later than the systole in the cardiogram and sometimes in mid-systole. It then continues to rise for a period slightly longer than the duration of the systole. This phase is constant in all thermocardiograms and since it is an invariable accompaniment of the systole, it may be referred to as the *systolic temperature rise,* or systolic heat production. It doubtless is associated with the intrinsic muscular processes that produce the normal systole of the heart.

2. The first negative oscillation, or *first temperature fall, phase 2.* This phase begins promptly at the end of the first temperature rise without any temperature stasis intervening. It begins usually a little after the onset, or even in middle of diastole as indicated by the cardiomyograph. It continues to show fall of temperature until near the end of diastole, its duration being usually a little greater than that of diastole. The magnitude of this phase of the thermocardiogram is remarkable in that it is greater than any other one phase. Indeed, being negative in sign, it is just great enough to cancel the positive number which is the algebraic sum of all the other phases.

Since this phase is an invariable accompaniment of the diastole it may be referred to as the *diastolic fall of temperature*, or the diastolic cooling. In seeking an explanation for this remarkable phase of the thermocardiogram, a number of factors must be given consideration.

1. The normal cooling following the cessation of systolic heat production accounts for some of the fall of temperature. But it cannot account for all of it. The fall is invariably much below the pre-systolic or resting temperature level. Furthermore its velocity is greater than one would expect if it were due to mere loss of heat by conduction and radiation. One must therefore seek additional causes.

2. One naturally would suspect a slipping of the thermopile junctions from warmer to colder points on the muscle surface. But this was a source of error that had been carefully guarded against. The method of suspending the framework for the support of the heart from the cover of the jar enables one to mount the ventricle and put the thermopile in position outside of the jar and thus in full view of the observer. One can thus inspect the ventricle halves and the thermopile during any number of beats and assure oneself that no slipping occurs so far as the eye can estimate it. By this procedure it was made certain, *a*, that the warm junctions of the thermopile were completely covered by the two halves of the ventricle; *b*, that the initial tension on the ventricle was great enough to cause its inner surfaces to hug closely the surfaces of the thermopile and also to diminish the actual shortening of the muscle during systole; *c*, that the flexible terminal lead wires from the thermopile were quite free and that the thermopile rode quite freely with the ventricle in case there was any movement on the part of the latter.

The method of recording the cardiomyogram did not admit of using a perfectly isometric lever. From the lever length, known points of muscle attachment, and the excursions of the writing tip the actual shortening of the ventricle length can be calculated in all the experiments. The following are typical:

DATE OF EXPERIMENT	ACTUAL SHORTENING
	mm.
11/19/20 (See fig. 1)	0.19
11/24/20 (See fig. 2)	0.89
2/26/21 (See fig. 3)	0.58
5/25/21 (See fig. 4)	0.42
6/ 5/21 (Not shown)	0.52

The length of the ventricle on the average was about 20 mm. The shortening of the muscle when nearly greatest therefore may be placed at about 1 part in 30.

But since the thermopile rides freely with the muscle mass and since the shortening proceeds probably from both ends toward the middle, and allowing 0.15 mm. as the maximum diameter of the junctions, it is not conceivable that the vertical displacement of contiguous surfaces between muscle and pile could have been in any case more than 0.003 mm.

In spite of this small and negligible vertical displacement there is still the horizontal displacement, that undoubtedly took place, to be considered. There are no data at hand from which the actual horizontal shortening of the ventricle can be estimated. The wires of the thermopile lie parallel with the lateral axis of the ventricle and the displacement of the surfaces in this case would be from junctions to wires,—to metal that by its rapid conductivity would probably have already taken on the same temperature of the junctions. Finally it must be admitted that the contraction along the horizontal axis will diminish shortening along the ventrical axis and in that way correspondingly diminish the error of vertical displacement. Taken all in all, therefore, the scource of error due to lateral as opposed to the base-apical contraction can not be considerable

3. The effect of elastic stretching and elastic recoil may now be considered. Steiner (7) and others (8) indicated a measureable thermoelastic effect in muscle when it is subjected to elastic stretching and release. In the present experiments it is conceivable that when the internal tension subsided, at end of systole, the recoil of the lever spring would suddenly stretch the heart walls thus producing a thermoelastic effect that would appear in the thermocardiogram. But as Engelmann (9) pointed out, some organic substances including muscle show thermoelastic effects just the reverse of those shown by metals. Hill and Hartree (10) have recently measured the cooling effect of the elastic recoil of frog's sartorius muscle that had been released from a stretching weight. From their data they calculate that the coefficient of thermal expansion is -10^{-4} to -10^{-5}, or that whenever heat it added sufficient to raise the muscle's temperature 1°C., the muscle will shorten under constant load one part in every ten or one hundred thousand parts. The thermodynamic converse of this indicates a rise of temperature on the muscle if put on an elastic tension. But according to the thermocardiogram it is just at this time we have no rise but a fall of temperature.

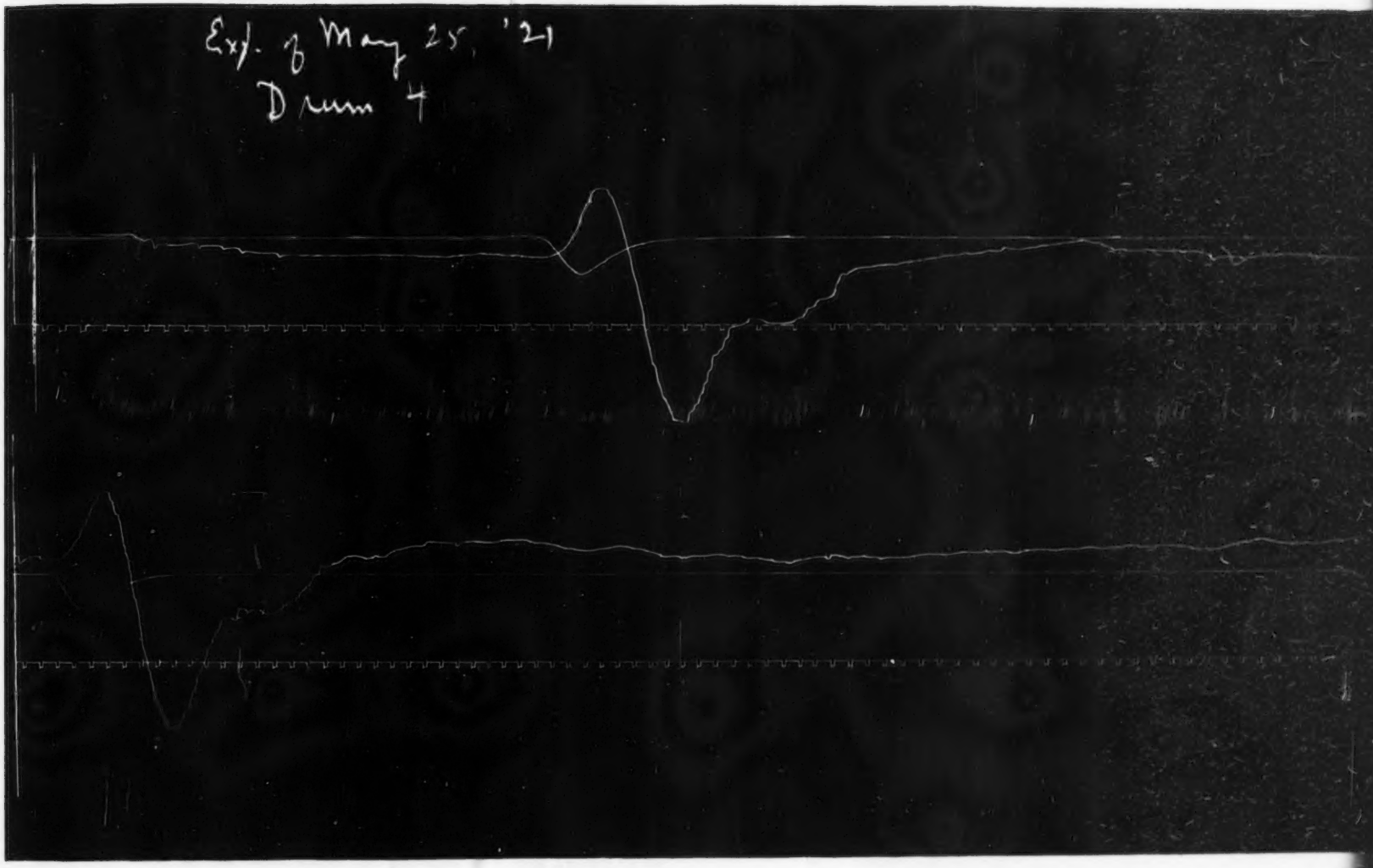
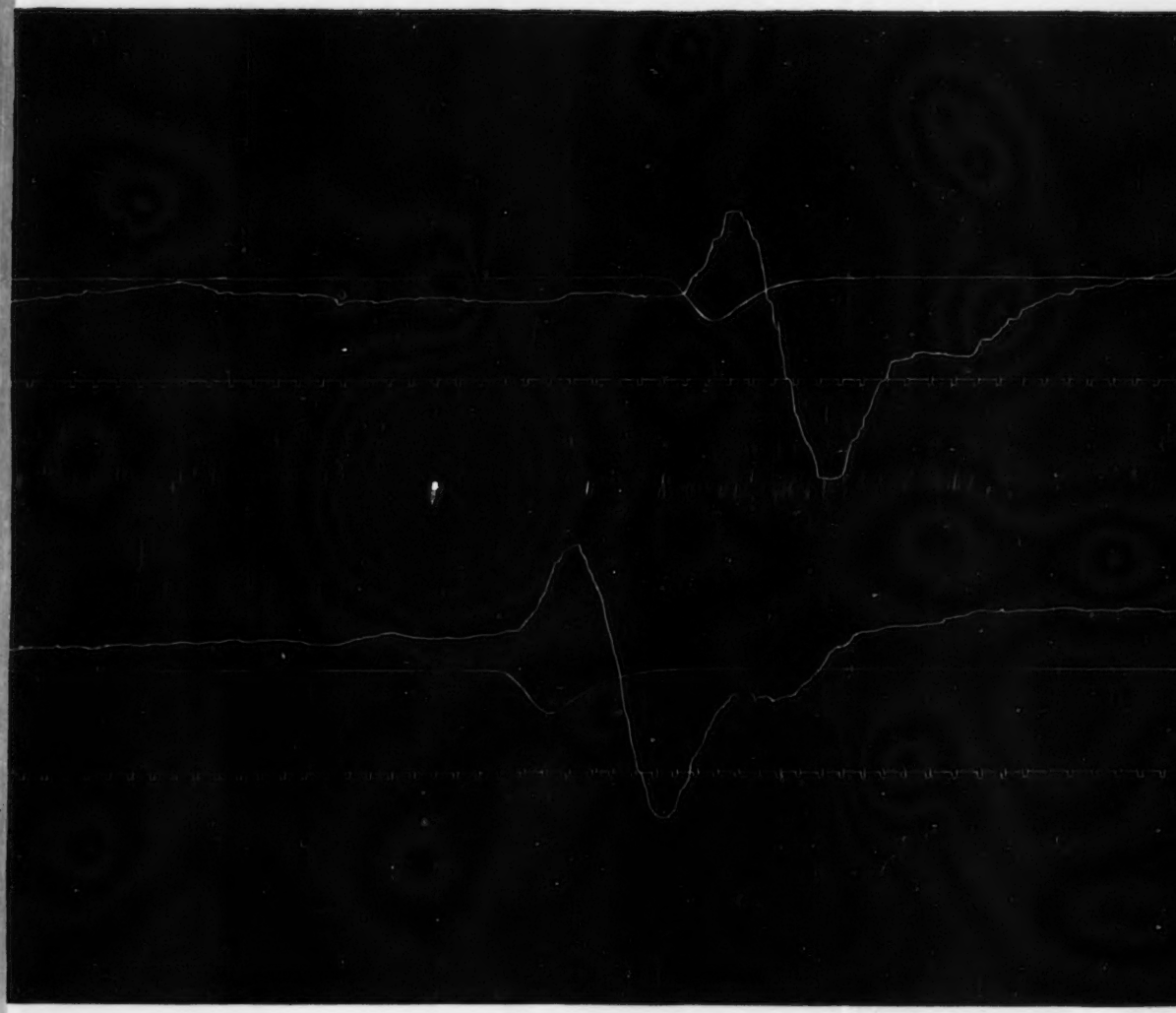
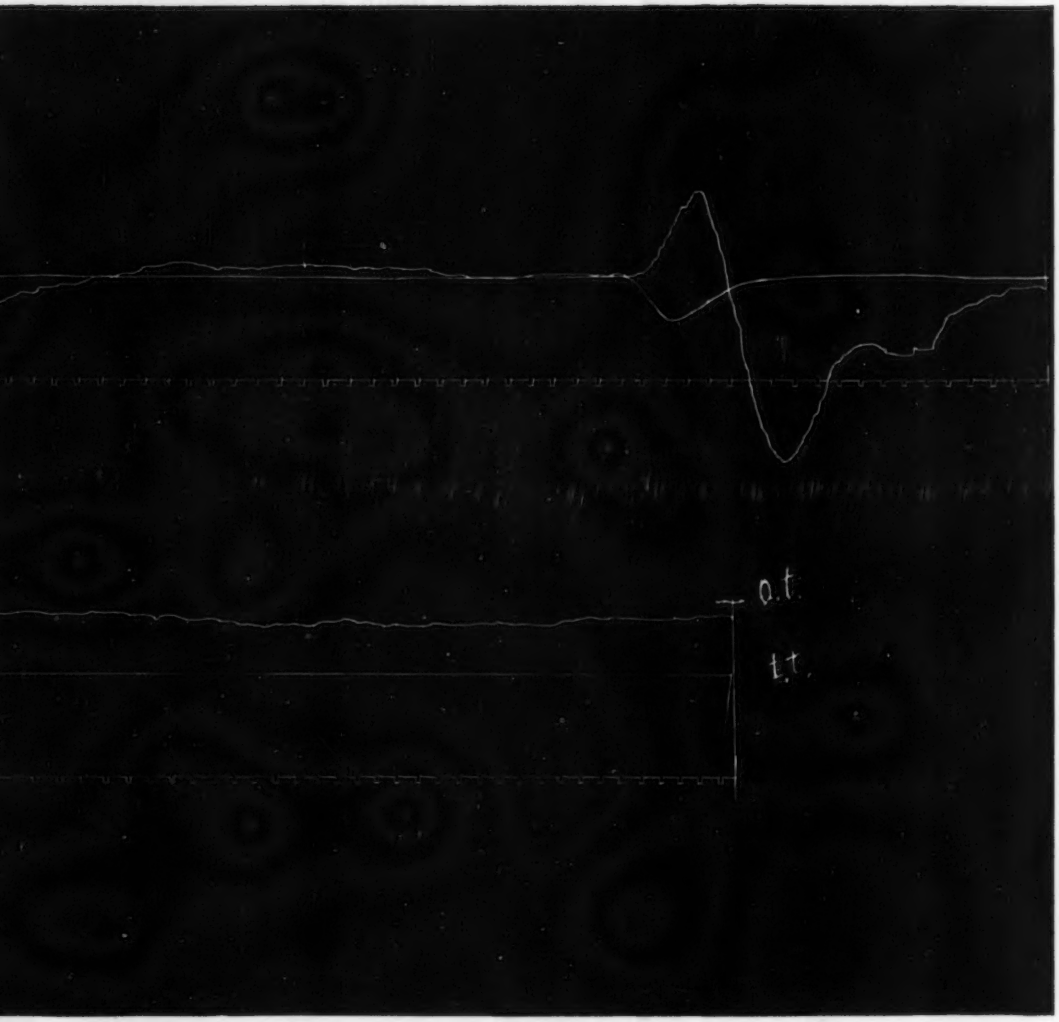


Fig. 4. An original drum record (no. 4) of the experiment of 5/25/21, showing the polyphasic thermocardiograms from a very slowl



the polyphasic thermocardiograms from a very slowly beating heart. For additional experimental data and analysis see tabl



nalysis see table 1 and page 267. Time always three-second intervals

Accordingly we are forced to conclude *a*, that during diastole changes go on within the muscle that cause the fibers to become longer so rapidly that the elastic recoil of the muscle lever is not able to put them in turn on an elastic stretch. Or *b*, that if there is some slight elastic tension developed there are other changes going on within the fibers, endothermic in nature, and of a magnitude more than sufficient to counterbalance its exothermic effect.

From the foregoing considerations it is concluded that the first or diastolic fall of temperature in the thermocardiogram is for the most part an expression of the actual change of temperature going on in the walls of the ventricle during a considerable part of diastole.

3. The second positive oscillation, or *the second temperature rise, or phase 3.* We now come to the second period of temperature rise. This phase follows promptly on the heels of the diastolic fall of temperature without a period of temperature stasis intervening. Since the diastolic fall is never completed until the diastole is over, this phase may be referred to as *the post-diastolic rise of temperature.* It is of greater extent and of longer duration than the systolic rise, but of less extent and of more nearly the same duration as the diastolic fall of temperature. It is never sufficient to bring the heart's temperature up to the pre-systolic level. It is always present at least as a distinct phase in the hearts beating so slowly as once in 30 seconds. Unless it can be shown that the diastolic fall described above is an artifact, it is difficult to see how this post-diastolic rise is in any way due to experimental error. The heart is completely quiescent when it occurs. It probably is due in part to equalization of temperature between the cold and warm junctions of the thermopile, by conduction along the wires. The time of this phase, *ca* 9 seconds, is great enough. Bürker (6, p. 289) says the flow of heat from cold to warm junctions in thermopiles such as I have used is negligible up to periods of 4.9 seconds. On the other hand, this post-diastolic rise of temperature must be due also in part to internal metabolism of the muscle.

4. The *fourth phase* of the thermocardiogram is somewhat varied in the different records. Sometimes, as in figures 2 and 4, it appears as *a temperature stasis.* In some cases it is a small negative oscillation. Only in the latter cases (experiments of June 5 and 10) have I been convinced that the negativity of this phase is due to periodicity of the galvanometer. In the cases where the galvanometer was known to be dead-beat there can be no doubt that it represents a genuine change in the heart's activity. During this phase it will be noted that the

heart is mechanically absolutely quiescent, and still it has not yet reached the pre-systolic temperature level. The phase as stasis or fall is present in all records where the beat is 30 seconds or more in duration, and is of special interest as an introduction to the next phase.

5. This phase of the thermocardiogram, *the third positive oscillation*, is only clearly present in records of hearts whose beats are 48 seconds in duration or more. It is therefore apparently absent in figures 1 and 3. Its duration is somewhat the same as that of the second temperature rise. In less slowly beating hearts it merges itself with the systolic rise of the next beat. But in the hearts where the beat is extremely slow (several minutes in duration) it is separated from the next beat by another phase.

6. The sixth phase is often a temperature stasis, but sometimes it is a period of slow rise, clearly a period of resting or basal metabolism. This phase is well illustrated in the long beat of figure 4, which has a duration of 230 seconds. The time of this phase obviously depends upon duration of the pause in the cardiac cycle, the one increasing with the other; the character also seems to depend upon the time of the pause, being more of a slow rise in the shorter pauses and a stasis in the longer pauses.

This completes the qualitative description of the various phases of the thermocardiogram, that are clearly concerned with the heart's activity. There appear from time to time lesser and irregular oscillations that are due to external factors disturbing the apparatus. These need not be considered here.

The quantitative time relations between the various phases of the thermocardiograms and temperature oscillations which they represent in terms of galvanometer scale divisions for two typical experiments, will be found in table 1. It has already been seen in figures 3 and 4 that the graphs of successive heart beats are much alike. Individual measurements in the table therefore also do not vary greatly, since they are made on successive beats of single drum records. The two hearts represented in the table are typical for the slower and faster rates of beats. It will be noted that the range of deflection for the whole beat is the same as the range for phase 2, or the diastolic fall.

Certain additional data should be given here from the protocols of the experiments represented in table 1:

In the experiment for 2/26/21 the ventricle weighed 1.45 grams. Analysis of the cardiomyograms shows that the initial tension on the ventricle was 14.4 grams; the maximum tension, 28.8 grams; the absolute tension, 14.4 grams; also that the

TABLE 1

Analysis of thermocardiogram phases

DATE	NUMBER OF EXPERIMENT	SYSTOLIC			DIASTOLIC		DURING PAUSE								RANGE OF DEFLECTION OF GALVANOMETER
		Lag	First rise, phase 1		First fall phase 2		Second rise, phase 3		Stasis or fall, phase 4		Third rise, phase 5		Stasis or fall, phase 6		
		sec.	*mm.*	*sec.*	*mm.*	*sec.*	*mm.*	*sec.*	*mm.*	*sec.*	*mm.*	*sec.*	*mm.*	*sec.*	*mm.*
May 25, 1921	3-1	1.5	12	9	44	10.5	19	12	0.0	7.5	12	27	0.0	78	44
	2	1.5	11	9	44	12	18.5	12	0.0	7.0	16	33	−3.0	69	44
	3	1.5	10	9	43	12	21	12	1.5	6.5	12	34	−3.5	70	43
	4-1	2.0	12	9	41	12	18	12	−0.8	7.0	15	49	−2.5	65	41
	2	2.0	13	9	40	12	19	12	0.0	7.5	14	33	−1.5	60	40
	Mean	1.7	11.5	9	42.5	11.2	19.5	12	+.35	7.0	14	38	−2.0	69	42
February 26, 1921	2-1	0.5	7.2	7.5	27.7	10	23.7	8	−3.0	4.5	None		None		27.7
	2	1.2	7.0	8.5	27.7	10	23.5	10	−3.0	4.0	None		None		27.7
	3	2.5	7.2	7.0	27.7	9	23.7	9	−3.0	6.0	None		None		27.7
	4	3.0	7.0	5.5	27.7	9	24.2	9	−3.0	7.0	None		None		27.7
	5	2.5	6.8	6.5	27.0	8.5	24	10	−3.0	4.5	None		None		27.0
	6	0.5	7.2	7.5	28.5	8.5	24.2	10	−2.8	3.5	None		None		28.5
	7	0.0	8.8	10.	30.	9	24.2	10	−3.0	6.0	None		None		30.0
	8	−0.5	8.8	9	29.	10	24.2	12	−2.8	5.5	None		None		29.0
	Mean	1.2	7.8	7.7	28.5	9.2	23.8	10	−2.9	5.2	None		None		29.3

duration of the systole varied from 5 to 6 seconds, of the diastole from 7 to 10 seconds, of the pause from 17.5 to 21.5 seconds, the total cardiac cycle varying from 32.5 to 35 seconds. The sensitivity of the galvanometer to 1 microvolt at 1 M distance was 26 mm. of scale; the period of the galvanometer, 16 seconds, the oscillation dead-beat. The mean range of deflection of the galvanometer during the thermocardiographic cycle being about 29.3 mm., the theoretical temperature deviation for the heart beat in this experiment is about 0.0007° C. The temperature of the heart chamber was 10.6°C.

In the experiment of 5/25/21 the temperature of the heart chamber was 9.0°C. weight of ventricle, 2.0 grams. Analysis of the cardiomyogram shows initial tension on the ventricle to have been 19.4 grams, maximum tension 28 grams, absolute tension 8.6 grams; duration of systole was always 6.5 seconds, diastole varied from 15 to 23 seconds, the pause varied from 107 to 128 seconds, the mean duration of the whole cycle was 142 seconds. The actual shortening of the ventricle at each beat was 0.42 mm. The performance of the galvanometer was as follows: sensitivity, 18 mm. to right and 17 mm. to left, and dead-beat. The difference between galv. null point and the heart's resting heat production was about 180 mm. of scale, the warm junctions being that much colder than the "cold junctions." The mean range of temperature deviation of the heart for each beat was 42 mm. scale divisions or about 0.0015°C.

The graphic records of a number of typical experiments of the investigation have been analyzed and the results tabulated as those of the two experiments shown above in table 1. The mean values in these various tables are brought together and shown here in table 2. In this latter table the phases of the cardiomyograms as well as those of the thermocardiograms together with the duration of the heart beat are brought together for comparison. Furthermore the phases of the thermocardiograms in order that they may be put on a more accurate basis of comparison have been expressed in terms of degrees centigrade, instead of the direct readings, millimeters of galvanometer scale division. The experiment of June 4 is given in two different stages, the earlier and later stages showing great differences of heart rate. The experiment of June 10 is also given in two groups; here the rate remains the same but the initial tensions in the two groups differ considerably.

In this table is added a column wherein the sums of the rises 1, 2 and 3 of the thermocardiograms are shown. This sum is greater than deviation of the first fall, in cases where there are subsequent falls. If these latter are added (phases not shown in the table) this difference disappears.

No attempt has been made at this time to carry out a systematic study of the exact relations between tension and heat production in the heart muscle. For this quite different methods will have to be em-

TABLE 2

Mean temperature differences in degrees C. for the various phases of the thermocardiograms in various experiments together with duration of heart rates and tensions,—corresponding cardiomyogram phases

DATE OF EXPERIMENT	NUMBER OF DRUM RECORD	PHASE 1, FIRST RISE, × 1000	PHASE 2, FIRST FALL, × 1000	PHASE 3, SECOND RISE, × 1000	PHASE 5, THIRD RISE, × 1000	SUM OF RISES, 1, 2, 3, × 1000	TIME OF BEAT, SECONDS	TENSIONS IN GRAMS			WEIGHT IN GRAMS, OF VENTRICLE
								Initial	Maximal	Absolute	
2/26/21	dr. 2	0.18	0.70	0.57	None	0.75	34	14.4	28.8	14.4	1.4
5/ 4/21	dr. 5, 6	0.12	1.3	0.75	0.35	1.22	57	15.7	25.3	9.6	2.8
5/25/21	dr. 3, 4	0.66	1.5	0.11	0.80	1.57	142	19.4	28 0	8.6	2.0
6/ 4/21	dr. 2, 3	0.49	1.29	0.87	Fused	(1.36)	41	17.7	29.6	11.9	2.7
6/ 4/21	dr. 3, 4	0.38	1.08	0.75	0.28	1.41	81–168	15.1	22.0	7.2	2.7
6/ 5/21	dr. 7, 8, 9	0.79	1.98	1.12	0.23	2.14	67	24.4	38.0	13.6	3.7
6/10/21	dr. 5,	0.19	0.74	0.48	0.17	0.84	48	35.7	45.0	9.3	1.5
6/10/21	dr. 1	0.16	2.17	1.72	0.67	2.55	48	8.0	20.0	12.0	1.5
Average.....		0.37	1.34	0.8	0.41	1.49		18.8	29.6	10.8	2.3

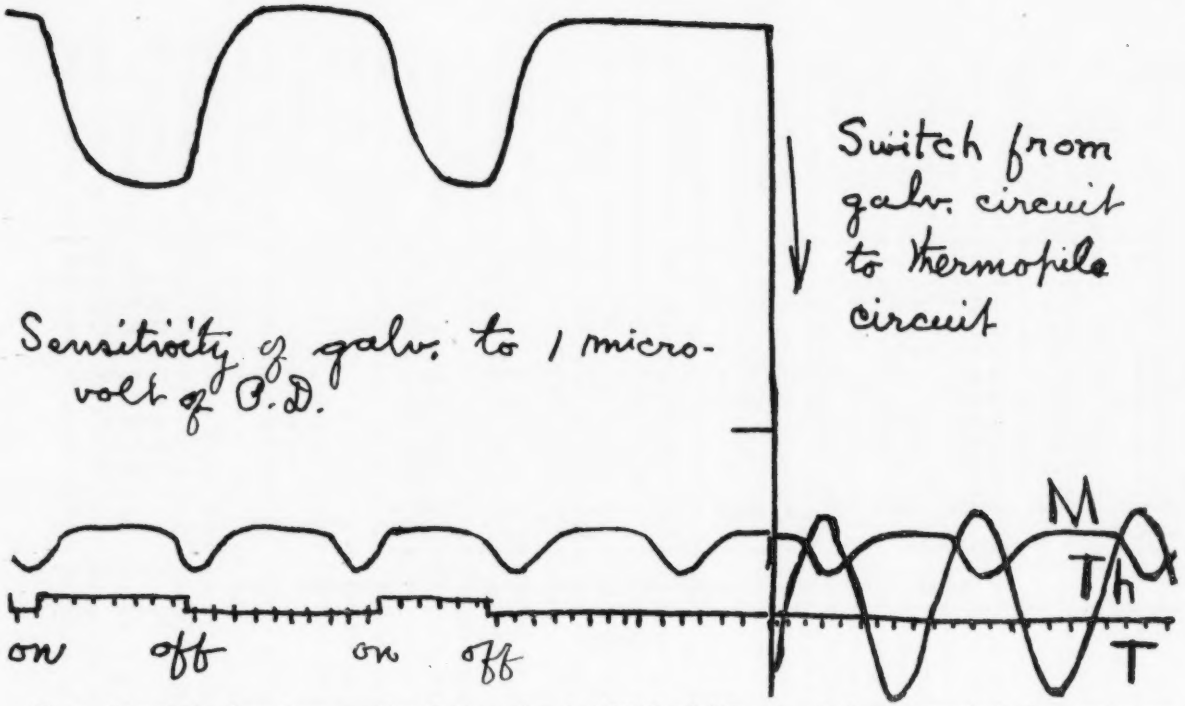


Fig. 5. Line tracing from drum record no. 2 of experiment of 5/4/21, slightly reduced, showing record of galvanometer performance,—response to calibrating current, dead beat swing; steadiness of null point; deviation, of the thermoelectric current of the heart, from the null point when the thermopile circuit is switched on to galvanometer. Finally the synchronous myo- and thermocardiograms, *M* and *Th*, are shown. Time, *T*, is always 3-second intervals. The duration of heart beat is 24 seconds giving here only diphasic thermocardiograms.

ployed. The heart-bag of Rhode (11) was employed with success in conjunction with a clasp thermopile (2), and it is probable that this will give best results in any tension study.

In table 3 the times in seconds of the chief phases of the thermocardiograms are given for comparison.

DISCUSSION OF RESULTS. 1. The results of this study of the detailed oscillations of temperature within the cardiac cycle in the first place corroborate those of the earlier investigations of this series (1), (2) in that *a*, the beginning of temperature rise occurs just with or slightly subsequent to the beginning of rise of tension in the muscle. Under the caption of "lag" in the second column of table 3 the data on this

TABLE 3

DATE OF THE EXPERIMENT	NUMBER OF DRUM RECORD	THE MEAN DURATION IN SECONDS OF THE PHASES OF THE THERMOCARDIOGRAM								TEMPERATURE OF HEART CHAMBER
		Lag	First rise	First fall	Second rise	Second fall	Third rise	Presystolic stasis	Whole heart beat	
										deg. C.
2/26/21		[+1.2]	7.8	9.2	10.0	5.2	(Fused)		34	[10.6]
5/25/21		+1.7	9.0	11.2	12.0	7.0	38.0	69	142	9.0
6/ 4/21	dr. 2, 3	[−1.5]	9.0	11.0	11.0	8.0	(Fused)		41	[8.0]
6/ 4/21	dr. 3, 4	+1.0	7.5	10.0	12.0	8.0	21.0	21	81–168	8.0
6/ 5/21	dr. 7, 8, 9	+0.2	5.2	8.8	8.0	8.0	9.5	27	67	12.5
6/10/21	dr. 5	+1.0	3.5	8.5	7.5	5.5	8.2	12	48	7.5
6/10/21	dr. 1	+1.0	4.7	10.2	10.5	5.5	10.2	11	48	9.0
Average		+0.78	7.1	9.9	10.3	6.9				9.2

point are brought together. In one case it will be noted that the rise begins apparently before systole, the lag being negative in sign. If one inspects the graphs in such cases it will be found that the presystolic portion of the rise belongs really to the preceding heart beat. In the more rapidly beating hearts the third rise of temperature of one beat may be fused with the first rise of the subsequent beat. In the still faster hearts even the second rise may be fused with the third and the third with the first of the next beat until one has a thermocardiogram such as one sees in figure 5, where the rise of temperature consists not only of one phase as in figure 1, but also begins apparently in middiastole. That in this latter case, the rise begins with the second rise of one beat and is fused with the first rise of the next, appears in the

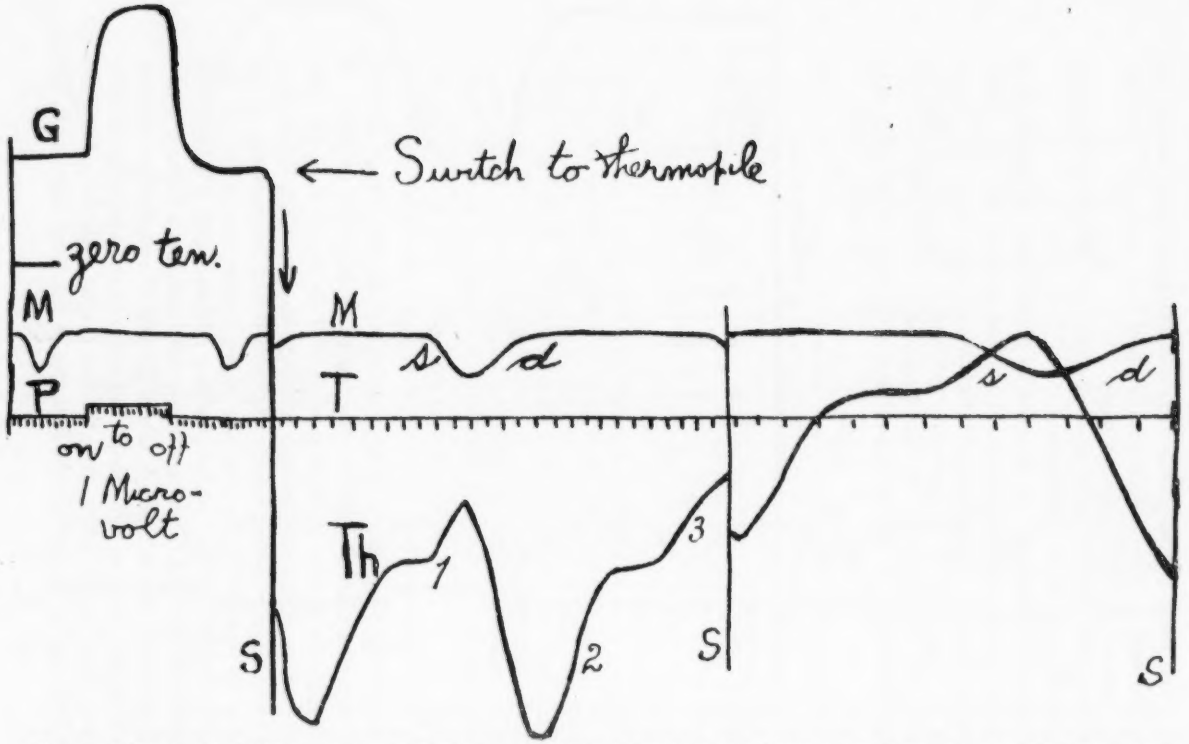


Fig. 6. Line tracing of drum no. 3 of experiment of 5/4/21, reduced. Same heart as in figure 5, somewhat later when the duration of beat is slower. The thermocardiogram now shows signs of going over into the polyphasic type. Performance of galvanometer is here again indicated.

records of this same heart when it is beating at a slower rate, as it does in figure 6. Here a distinct stasis appears in the thermocardiogram before the systole begins and the first rise begins to be sharply marked off from the post-systolic rises of the preceding beat.

This fusion of phases may produce still another picture. The rate may be such as to cause the second fall of temperature to occur just as the first rise of the next beat comes along. The result is a greater lag than usual.

To give the onset of heat production its truer position in the sequence of cardiac events it now will be necessary to add to this lag the latency of the mechanical response itself to the inner stimulus that falls upon the muscle substance. From records of experiments in 1909, the data of which were not then fully published (12), I find ten observations of latency of terrapin non-beating ventricle to direct electrical stimulation. These latencies varied from 0.4 to 0.96 second, and were observed at temperatures varying from 8° to 11.5°C. The mean values of these numbers would place the latency in question at 0.68 second for a temperature of 9.7°C. Assuming now that in the present case the spontaneously beating hearts had a latency to the inner stimulus of a magnitude similar to the one for non-beating ventricles, we need only add the "lag" of the thermocardiogram appearing in table 3 to get an approximation of the full latency. I take then 0.68 + 0.78, or 1.4 seconds as this approximate latency of the terrapin's thermocardiogram to the intrinsic stimulus, as it can be measured by the present method. The obvious corrections, reckoned out of specific heats and conductivities of heat, both of muscle and thermopile, as well as the delay of mechanical levers, should be made when ever the importance of the phenomenon demands it. In all however we may be confident that they will not cancel the whole of the figure, 1.4, and we may safely conclude that *heat production in the ventricle begins not earlier than the onset of tension rise.*

2. The next point in the former studies that has been corroborated by the present is *b, the range of temperature deviation during the caridac cycle.* In the former studies this range was 0.0013° and 0.0018°C., respectively. As will be seen in table 2 (compare columns 3 and 6) the average temperature deviation in the present experiments *is 0.0014°C.* The agreement in these three separate determinations is of special interest since a different type of galvanometer was used in each, a Paschen, an Einthoven and a d'Arsonval. I now proceed to the points that have been specially brought out by the present investigation.

3. *The systolic rise of temperature.* This phase of the thermocardiogram beyond all doubt represents a production of heat on the part of the heart muscle that is intimately associated with the normal processes of tension development. Among the chemical reactions that have been associated with rise of tension is the sudden and facile formation of lactic acid. The best proof we have of this formation is the fact that the minimal quantity of lactic acid is only found when every precaution to prevent excitation of the muscle to be analyzed is observed (13)(14). If now following immediately upon excitation this reaction, say by increasing the CH of the muscle substance, results in a sudden change in the elastic property of the mass, so that in the unstretched condition it would have a length less than it had before the excitation, one can readily see how the muscle, either in its normal position in the body or under the conditions of experiment, would not be able to take on its new length without meeting resistance, and that this resistance would put the muscle on an elastic strain. As was stated above, it is known that muscle belongs to that group of substances having a negative coefficient of expansion. Accordingly the stretching tension created during rise of tension or by the excitation would be an exothermic process, and result in rise of temperature.

The first rise of temperature in the thermocardiogram may be looked upon therefore as being in part of thermoelastic origin. The rise in this phase, according to table 2, averages a little less than 0.0004°C. Hill and Hartree (10) have shown that "a load of 150 grams added to a pair of sartorius muscles of Rana temporaria, weighing say, 150 mgm., would raise their temperature by something of the order of 1 to 3 thousandths of a degree, this being the fifth to a half of the rise produced by a strong twitch." Referring now again to table 2, one sees that the systolic rise on the average is about 27 per cent, somewhat more than a fourth, of the average total deviation of temperature for the beat, which we may consider comparable in this case (hearts beating all or none) to a strong twitch. But the loads and the weights of the ventricles differ greatly. If we ignore weights (since calories increase with muscle weights) for the moment and assume that the thermoelastic effect diminishes with load, then, since the average absolute tension my hearts exerted was 10.8 grams, the thermoelastic effect one may expect would be of the order of 0.6 or 1.8×10^{-4}. Or if the effect follows more nearly the maximum tensions, the thermoelastic rise would be of the order of 0.00018°C. or 0.00054°C. This latter figure brings the possible thermoelastic effect so near to the

systolic rise as it appears in the thermocardiograms that it seems unnecessary to look elsewhere for its explanation. This presentation of the case indicates that experiments must be carried out to furnish data that will be more applicable to the ventricle and upon which one may base one's calculations with more confidence before a final conclusion can be reached.

It should also be pointed out that Bürker (6) has shown that a period beyond 5.2 seconds is no longer negligible as to the flow of heat from the warm to the cold junctions of his thermopiles. Since the period of systolic rise is 7.2 seconds some equalization of temperature between junctions however slight has doubtless taken place. Furthermore the next phase is negative and comes on so suddenly that it appears to cut short the wave of systolic rise.

TABLE 4

Duration of the various phases in the cardiomyograms in seconds

DATE OF EXPERIMENT	RECORD NUMBER	SYSTOLE	DIASTOLE	PAUSE	WHOLE BEAT
2/26/21		5.5	8.5	18.5	34
5/25/21		6.5	19.0	117.0	142
6/ 4/21	pt. 1	4.5	12.0	25.0	41
	pt. 2	5.0	11.0	65–152	81–168
6/ 5/21		3.2	6.2	58.0	67
6/10/21	dr. 5	4.0	6.5	38.0	48
	dr. 1	4.0	9.5	35.0	48
Average		4.67	10.4		

4. The diastolic fall of temperature in the thermocardiogram as pointed out above may be due in part to two different causes. The duration of the phase is even greater than that of the systolic rise so that here again we would expect some equalization of junction temperature by conduction of heat along the wires from the warm to the cold junctions. Some small amount of heat will also be lost by conduction and radiation from muscle to air. But both of these causes could only bring the galvanometer spot toward the pre-systolic level. The curve in the thermocardiogram however sweeps down farther beyond this level than the systolic wave had reached above it. Furthermore the steepness of the curve leaves no doubt that a rapid cooling of the warm junctions is taking place, a cooling too rapid to be explained by mere conduction and radiation either between muscle and air or between the two sets of junctions. The duration of this phase is not always

the same as that of the diastole, or the fall of tension, although the average numbers (9.9 and 10.4) are very nearly so. (Compare tables 3 and 4.) Furthermore while the onset of diastolic fall of temperature is always a little later than that of fall of tension, it continues on beyond the end of the latter correspondingly.

It will be remembered that in the critical analysis of the results above the possibility of this fall of temperature being due to experimental error was answered in the negative. This phase of the thermocardiogram therefore must be regarded as being intimately associated with the processes concerned with fall of tension within the heart muscle and therefore with diastole. It thus becomes of interest to inquire further as to what endothermic processes are involved during fall of tension in muscle.

It has just been shown that during systole there may be produced in the muscle an elastic strain, and that this in turn would give rise at least to some of the heat that causes the systolic rise in the thermocardiogram. The diastole of the heart may be looked upon as reversal of the systolic processes. If so then the processes underlying it may also be suspected as being in part at least reversals of the underlying systolic processes. Since the production of elastic strain has been hypothecated during the increase of muscle tension, its reversal, or elastic recoil, may be looked for during the decrease of muscle tension. This will be clearer if we think of the relaxation of muscle, the lengthening of the muscle mass, as the releasing of a stretching weight from a stretched elastic body. During the contraction the muscle becomes suddenly a shorter body, but being held between the same two fixed points it is put, or puts itself, on an elastic tension. During relaxation the muscle suddenly becomes a longer body that equally suddenly finds itself reaching across from one fixed point to another without any elastic strain, a change that in all essentials is nothing more than an elastic recoil. Elastic recoil being an endothermic process, heat from the surroundings would be absorbed and a fall of temperature should be expected. The thermocardiogram shows a fall of temperature during diastole, during muscle relaxation, and it is therefore concluded that the thermoelastic effect of relaxing muscle is an additional cause of this fall.

5. The post-diastolic rise of temperature, the second rise, or the third phase of the thermocardiogram, has a duration of nearly the same number of seconds as the diastolic fall; in extent however it does not swing beyond, but rather stops short of, the presystolic level.

Lasting as it does from 7 to 12 seconds (see table 3) it doubtless is in small part due to transfer of heat from cold to warm junctions and to absorption of heat by the cooled tissue from its surroundings. But it is also possible that this phase is due again in a small amount to another thermoelastic effect. Upon the fall of tension in the muscle there follows the elastic recoil of the isometric lever spring and this in turn, since the recoil is not to zero tension of the lever but to "initial" tension, will put some of the heart tissue on an elastic strain. More probably in this case the connective tissue or even the sarcolemma rather than the cotractile substance is affected. In any event there will again come into play a positive thermoelastic effect and rise of temperature.

One other source of heat may have its effect, one that until now has not been mentioned. I refer to heat of chemical reaction. All the recent work on the chemistry of muscle contraction points to the period after the contration as the one in which oxidation takes place, that the period of contraction is one of anoxybiosis and that the period of oxybiosis only begins after the contraction is over (14). This post-diastolic rise therefore may be looked upon as in part also due to heat of oxidation. The velocity of this reaction in my hearts and the heat production therefrom was probably much slower than the heat production of the thermoelastic effects.

6. The fourth phase. This as already pointed out may even be a slight fall in temperature; it lasts from 5 to 8 seconds. This phase will be better understood in the light of what will be said later on under the head of general discussion.

7. The fifth phase of the thermocardiogram or the third rise of temperature. This rise can be ascribed with certainty to the continued heat of oxidation. If the duration to the next heart beat is long enough this oxidation comes to a standstill, and we have the final significant portion of the thermocardiogram.

8. The sixth phase, or the period of pre-systolic stasis. This phase I take as an expression of the resting metabolism of the heart muscle. The heat produced is no more than is lost by conduction and by transfer from warm to cold junctions, and will continue indefinitely if no new beat of the heart comes along to disturb it. In figure 4 are shown excellent illustrations of this phase in the slowly beating hearts.

9. Interpretation of phases in beats of short duration. With this picture in mind it will be readily seen that, as the heart's rate increases, it will be the last phase, the period of pre-systolic stasis of the thermo-

cardiogram that will be the first to be cut short in point of time. And as it is well known that of all the phases of the cardiac cycle it is the pause that is sacrificed in the process of shortening the duration of the cycle, so it must follow that the other phases of the thermocardiogram that happen to fall within the period of the pause will be sacrificed. In cases where the rate of beat is caused by an increase in temperature there can be no doubt that the primary influence of the temperature up to certain limits is on the inner-stimulus to beat. The period between excitations is diminished. It has further been shown that the reduction of this period follows the van't Hoff rule for chemical reaction velocities, a beautiful demonstration of which has been given recently by Garrey (15). This being the case, it is clear how the excitation will fall finally at the end of the third rise, so that the third rise of one heart beat becomes fused with the first rise of the succeeding beat. This seems to be the case in figure 3. And since the stasis between the second and third rises is due to a sluggish chemical reaction (oxidation) in the slower rate of beat, it is clear how this reaction will be hastened with temperature increase also according to the van't Hoff rule so that this stasis in the thermocardiogram also gradually disappears with rise of temperature and the second phase becomes fused with the third phase. We have then finally a thermocardiogram of diphasic character such as is shown in figure 5 (right hand lower section).

From the discussion in the preceding paragraph it is clear that the limb of the curve representing rise of temperature in the diphasic thermocardiograms is a fusion of three distinct phases of heat production. Beginning with the end of the phase of fall they are in order, the second rise, the third rise and the first rise, as they would occur in the polyphasic thermocardiogram. These diphasic graphs ought therefore to have their curves of temperature rise beginning with end of diastole, or at some pre-systolic time, rather than with rise of systole as they do in the polyphasic graphs. This is the case in the graphs in figure 5. The fall of temperature, on the other hand, ought to occupy about the same place in the diphasic as it does in the polyphasic thermocardiograms. This also is the case in the record shown in figure 5.

The diphasic temperature graphs of figures 5 and 1 seem to contradict each other. The seeming contradiction is explained by the difference in heart rate of the two. In the former the rate is 27 seconds, in the latter 15 seconds. In the one the rise of temperature is in the pause. It is not difficult to see how this rise may be inaugurated by the second rise of the polyphasic graph. But in figure 1 the rise begins with systole,

according to the mechanical lever tracing. Is this rise not clearly inaugurated by the first rise of the polyphasic graph? I have concluded not. The brief time interval has simply crowded the rise of the diphasic graph apparently over into the systole. Its initial upshoot is still the second rise of the polyphasic followed by the third and then as a cap to the anacrotic limb we have the first or systolic phase of the polyphasic thermocardiogram. I have indicated this analysis of the rise in the diphasic thermocardiogram of figure 1 with the numbers *2, 3* and *1*, for the 1st and last heart beats in the figure.

GENERAL DISCUSSION COMPARING THE PHASES OF HEAT PRODUCTION WITH THE PHASES OF CHEMICAL REACTION IN MUSCLE

Until now I have put off discussion of the heats of chemical reactions as a source of heat production other than a mere statement of the oxidative reaction. The nature of the chemical reactions involved in muscle contraction is now known, thanks to the recent work of Meyerhof (14), in sufficient detail to warrant an examination of their bearing upon the results of the present investigation.

This work of Meyerhof has crowned a long series of researches by many authors as well as his own with brilliant success. It is now certain that following close upon excitation and during contraction some muscle glycogen, as many older physiologists had supposed, undergoes hydrolytic cleavage into dextrose. Dextrose then promptly undergoes further splitting into lactic acid. During the period of recovery the lactic acid thus produced completely disappears in the ratio of one part oxidized to CO_2 and H_2O, to four parts built back into glycogen (14, III and IV). These results of Meyerhof are based upon the balance sheets of the analyses covering long experimental periods, of muscles after being tetanized for a half-hour or longer, and of others that were allowed to recover for as long as a day. It would seem far-fetched to expect the changes found in these long periods to be an exact counterpart of what happens during the short period of a single muscle action or the period of a single cardiac cycle. But when one remembers the incessant action of the heart *in situ* it must be admitted that whatever period of recovery falls to its lot must fall somewhere within the limits of each beat, and that this recovery must be complete for the effects of the work that fall within the limits of that same cardiac cycle. This has been a dictum among physiologists for a long time, but it is necessary to restate the thought to justify the correlation that I wish now to make between the metabolism find-

ings of Meyerhof and other observers, and the various phases of the thermocardiograph as I have found them to be.

In one of my former papers (2) I briefly pointed out that the heat production of 1 gram of heart muscle per beat approximated closely the amount demanded by the sugar metabolism studies made on isolated beating hearts. In the metabolism studies the amount of carbohydrate utilized is given per hour. But wherever weights of the hearts, rate of beat, etc., are given I could calculate the proportional amount of carbohydrate per gram of muscle per beat. In one or two cases the weight of the heart or the rate of beat had to be estimated. The parallel is so striking and important however, that I shall put the figures down of a few cases again with the sources of the original data.

Carbohydrate utilized by surviving heart muscle per gram muscle per beat

OBSERVED BY	DATE	GRAMS $\times 10^7$
Müller	1904	2.66
Rohde	1910	3.0
Knowlton and Starling	1912	3.7
Underhill and Prince	1914	2.5
Average		2.96

The heat equivalent of the average weight of dextrose given in the column above is $677.2/180 \times (2.96/10^7) \times 1000 = 0.0011$ sm. calories. In my experiments, like the muscles in Meyerhof's work, the hearts were not perfused but drew on their store of glycogen for carbohydrate. But a weight of glycogen equivalent to the dextrose would have the same heat value. From the average figures in table 2 it will be seen that the sum of the rises of temperature in the thermocardiogram is 0.00149°C. This number times the specific heat of muscle, 0.825, would represent a heat production per beat of 0.0012 small calorie. For reasons pointed out above (see discussion of results) the range of temperature represented by the second phase of the thermocardiogram, the first fall, is probably a nearer measure of the correct total heat exchange than the sum of the rises. The average of this phase, 0.00134°C. times 0.825 is 0.0011 calorie, a number identical with the heat equivalent of the average carbohydrate consumption in the metabolism experiments quoted above.

According to Meyerhof's researches there is no longer any question but that it is not only carbohydrate but specifically glycogen that is

drawn on by surviving frog's muscle for energy. It is most probable that glycogen is also the starting point in the normal activity of heart muscle, and just as Meyerhof has shown that there is a partial resynthesis of glycogen in the recovery of frog muscle, so we may be confident that the same resynthesis occurs in heart muscle. Especially does this assumption appear all the more probable when we recall the great tenacity with which frog hearts *in situ* maintain their supply of glycogen as shown by the experiments of Jensen (16). Furthermore there is no longer any question, but that the chain of chemical events that covers the span of a muscle action, or of a cardiac cycle, is made up of two parts, an "anoxidative phase" and an "oxidative phase." What now are the heats of reaction involved in the chemical reactions that may make up each of these phases? And how do they compare with the heat production represented by the chief phases of the thermocardiogram?

The progress of the chemical reactions during contraction and recovery as deduced by Meyerhof in his latest work (14, III and IV) is as follows:

I. For the anoxidative stage,

$$^5/_n(C_6H_{10}O_5)_n + 5\ H_2O \rightarrow 5\ C_6H_{12}O_6 \rightarrow C_6H_{12}O_6 + 8\ C_3H_6O_3$$

This represents the reactions from, say, the moment of stimulation to the time when the maximum tension within the muscle is reached.

II. For the oxidative stage,

(1) $C_6H_{12}O_6 + 8\ C_3H_6O_3 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + 4\ C_6H_{12}O_6$

(2) $\rightarrow 6\ CO_2 + 10\ H_2O + {}^4/_n(C_6H_{10}O_5)_n$

The heats of reaction represented by the above chemical changes in terms of molecular equivalents may be summed up as follows:

I. For the hydrolytic cleavage of glycogen into dextrose,

$$(5 \times 679) - (5 \times 674) = + 25.0 \text{ calories}$$
$$\text{glycogen} \qquad \text{dextrose}$$

The cleavage of dextrose into lactic acid,

$$(4 \times 674) - (8 \times 329.7) = + 58.4 \text{ calories}$$
$$\text{dextrose} \qquad \text{lactic acid}$$

Total for the anoxidative stage, + 83.4 calories.

II. For the oxidative stage there is first the total oxidation of a molecular weight of dextrose, + 674.0 calories, and further the condensation of eight molecules of lactic acid back into dextrose,

$$(8 \times 329.5) - (4 \times 674) = - 58.4 \text{ calories}$$

Four molecules of dextrose finally undergo condensation with loss of water to glycogen,

$(4 \times 674) - (4 \times 679) = -20.0$ calories. A fourth loss of heat by the electrolytic dissociation of the CO_2, six molecules, at the rate of -2.8 cal. per molecule, $= -16.8$ calories. The algebraic sum of the heat exchanges for the whole of the oxidative stage thus becomes $674 - 58.4 - 20 - 16.8 = 578.8$ calories. Combining the totals for both phases we have the number, $83.4 + 578.8 = 662.4$ calories for the algebraic sum of the heat excess for the entire muscle action.[1]

It will now be of interest to compare this detailed oscillation of heat production, as demanded by the progress of the chemical reactions involved, with the detailed oscillation of heat production during the muscle action as shown by the thermocardiogram. To do this we have only to convert the readings in degrees of temperature into terms of calories, that is to multiply the readings by the specific heat of muscle, 0.825. This will give the quantity of heat per gram of muscle,—a standard of comparison. For it is plain that if the whole of the chemical heats of reactions are used up in producing the physical change leading to the final elastic tension, in a perfectly isometric contraction the systolic rise of the thermocardiograms ought to represent an amount of heat no greater than that demanded by the chemical transformations. Since as a matter of fact the heat of the systolic rise is more it must be concluded that the heat of anoxidative reactions is more than is necessary for the development of the elastic tension. Treating the averages found in table 2 in this manner we have the results as follows:

Heat production during the various phases of the thermocardiogram per gram muscle.

PHASE	TEMPERATURE IN °C.	HEAT IN SMALL CALORIES
First, or systolic rise	0.00037	0.0003
Second, or post-diastolic rise	0.0008	0.00066
Third rise	0.00041	0.00034
Sum of average rises	0.00158	0.00130
Average of total individual rises	0.00149	0.0012
First, or diastolic fall	0.00134	0.0011

In the analysis and discussion of the experimental results it was pointed out that the range of temperature represented by the second

[1] These heats of reaction are taken from Landolt's Tabellen, 4th edition. There are sources of heat exchange (Wärmetönung) other than those included in the inventory above. But they are small and largely neutralize each other. The final source of heat loss is the dehydration of the dextrose as it goes over to glycogen,—3.5 cal. per mol. wt.: another source is in the gaseous exchanges between muscle and air!

phase, or first fall in the thermocardiogram, was equal to, and therefore more probably nearer the algebraic sum of the heat exchanges during the whole of a heart beat. I therefore shall take this latter figure as the measure of the excess of heat for the whole thermal cycle, 0.0011 calorie.

Of the whole thermal cycle let it be assumed that the first or systolic rise covers the period of anoxidative chemical reactions. This in the thermocardiogram is 0.0003 calorie and has a ratio to the whole cycle of 3/11, or 1/3.66. The ratio if taken from the heats of chemical reactions as calculated above is 83.4:662.4, or 1:7.95.[2]

Does this difference in ratios between the observed heat production and that demanded by the chemical reactions involved indicate that heat arises from scources other than those of chemical reaction?

If this excess amount of heat in the systolic phase is due to the thermoelastic effect added to the heats of chemical reaction, we may proceed to calculate its magnitude from certain data at hand. For in that case we have the simple equation, in which x is the heat of thermoelastic origin, $1/8 = (0.0003 - x)/0.0011$, whence $x = 0.000162$ cal. This value can be converted into grams tension of elastic strain if one makes further use of Hill and Hartree's data (10) and supposes that the temperature rise, t', per gram load and gram weight of muscle is expressed by the equation,

$$t' = \frac{\text{observed rise in temp. times weight of muscle}}{\text{load on muscle}}.$$

Substituting then we have,

$$t' = \frac{0.001 \text{ or } 0.003 \times 0.15}{150} = 1 \text{ or } 3 \times 10^{-6}\text{°C}.$$

The thermoelastic effect as reckoned above may now be put in terms of degrees temperature by dividing by the sp. ht. of muscle, $0.000162/0.825 = 196 \times 10^{-6}$°C. From which it would follow that the average thermoelastic effect in the thermocardiograms had a value of 65 or 196 grams tension. The maximum tension actually observed during the experiments as shown in table 2 is 45 grams and the average is 29.6 grams.

[2] If one uses Berthelot's figures for heats of combustion this ratio is 1/5. I chose Stohmann's as being probably determined under better experimental conditions.

During the development of elastic strain in the muscle substance following excitation it is quite reasonable to suppose that the tension would be transmitted to the attached lever, and it in turn would respond, index hand of a spring balance that it is, to give a more or less accurate measure of that tension. Comparing the observed tension with the calculated values, and remembering the denseness of the jungle of speculation we now have reached, the closeness of the two figures is certainly more impressive than their disparity. Indeed this result is highly encouraging for a systematic investigation of the exact quantitative relations existing between tensions and thermoelastic effects in muscle.

The phases of the thermocardiogram covering the chemical reactions during the oxidative stage of muscle activity may now be compared. As we have seen, the algebraic sum of the heats of reaction during this oxidative stage is 578.8 cal. The ratio of this number to the sum of the heats for both anoxidative and oxidative stages is 578.8/662.4, or 1/1.14. Of the heats of this stage as indicated by the thermocardiogram we may take the sum of the second and third rises of the thermocardiogram oscillations. The ratio of this sum to the number representing the heat oscillation for the whole heart beat is 0.0010/0.0011, or 1/1.10. The approximation of the ratios of the heat of the oxidative stage to the heat of the whole muscle cycle, as determined by the two methods, is thus quite satisfactory.

It will be noted that in this treatment I have assumed the oxidative stage to cover both of the post-diastolic rises in the thermocardiogram. The question now arises as to what the meaning may be of the stases or falls that occur after each of these rises. It will be remembered that during the oxidative recovery, referring again to the sequence of chemical events, a part of the lactic acid is condensed back into dextrose, an endothermic reaction, and the dextrose thus formed is later still further condensed to glycogen, another endothermic process. Now Meyerhof in his latest work (14, III and IV) has shown that both of these processes are very intimately bound up with or rather dependent upon the process of lactic acid oxidation. On the face of the evidence it is certain that the formation of glycogen is subsequent to the formation of the dextrose, and that both processes depend upon certain optimal concentrations of the substances leading to the reaction, as well as a ready supply of heat to accelerate the reactions. These conditions would involve most likely measurable durations of time.

We may then picture to ourselves that in the oxidative stage of muscle action the oxidation of lactic acid first proceeds to the point

of furnishing sufficient heat to accelerate the synthetic reaction, lactic acid into dextrose. This reaction now not only absorbs heat, but what is more effective, removes lactic acid from the field of the other concomitant reaction, oxidation into CO_2 and H_2O, or in other words reduces the concentration of the acid to the point where its oxidation is greatly retarded. This in turn retards the rate of heat production resulting sometimes in a fall, more often in a stasis, of the oscillations of the thermocardiogram. The change of one moiety of the lactic acid into dextrose having been accomplished the accumulation of heat from the oxidation of the other moiety now proceeds for a time probably to a point of final equilibrium, and a rise of temperature of lesser extent results, when the last endothermic reaction sets in, the change of dextrose into glycogen. This produces a second stasis in the thermocardiogram, or even a fall, introducing the final or sixth phase.

This assumption that the glycogen formation is delayed to the second stasis is strongly supported by observations that under certain conditions glycogen formation is accelerated and glycogenolysis retarded by a *milieu* of lower C_H (17), (18), (19).

The general significance of the second phase, or diastolic fall, of the thermocardiogram has now still to be given consideration. From the general discussion up to this point it will be noted that the principal steps of the chemical reactions have been assigned to other phases in the thermocardiogram. The final reaction during the contraction is probably formation of lactic acid and no doubt is completed before relaxation begins. Physico-chemical effects such as the electrolytic dissociation of lactic and phosphoric acids may play a rôle during the period of relaxation; furthermore, neutralization or buffer effects may also be expected. In the absence of exact data in regard to them we can not even make an estimate of their magnitudes. They probably are small effects relatively, and it is known that their heats of reaction are often of opposite sign; for instance, the heat of electrolytic dissociation of carbonic acid is -2.8 cal. per mol. whereas that of phosphoric acid is given as $+2.4$ cal. per mol. The steps of the chemical reactions as given above from Meyerhof of course include only the most important ones. If one writes down all the probable intermediate physico-chemical reactions one finds there are no less than a dozen, each of which has its own heat of reaction.

In trying to account for this diastolic fall in the thermocardiogram I can only refer again to the more purely physical events that contribute. It is needless to repeat a description of them here. I only wish to

point out that the endothermic effect of the elastic recoil, as calculated above, is greater than I had at first thought it would be. On the other hand the loss of heat through conduction and loss of thermoelectric effect by transfer of heat from cold to warm junctions during this phase of the thermocardiogram can not be more than in other phases of like duration. Falls of temperature, therefore, through these causes are of less magnitude than I had at first thought, and should be given a place of less importance.

For the present then the fall of temperature during the relaxation of the heart muscle remains for the most part an enigma, and can only be cleared up by future investigations.

Finally it will be of interest to compare the results of this investigation with those of Hill and Hartree (20) on frog's sartorius muscle. In doing this one must bear in mind the difference in the muscle used, in the scource and kind of the exciting stimulus, and in the relative ease with which the results of the two investigations lend themselves to analysis and interpretation. The observers of the sartorius muscle find that following short lasting tetanic and direct stimulation heat is developed in three distinctly separated stages, one during the rise of muscle tension, one during the later stages of relaxation and one after the relaxation is over. The authors refer to this as three phases of heat production. Obviously there are stases if not falls of temperature of the muscle following each of these phases. A thermomyogram of sartorius therefore, as in the case of the ventricle, would be polyphasic, and indeed one of six phases as I have described for the heart muscle. The great difference between the two thermomyograms is the fact that in the case of the sartorius no fall of temperature, to say nothing of a stage of heat absorption, has been observed at any point, whereas a definite stage of heat absorption must be ascribed during relaxation in the case of the heart muscle. A smaller difference in the two myograms is in the position of the second period of heat production. In the sartorius this occurs "during the later stages of relaxation," in the heart muscle it occurs just as relaxation is completed. In brief, the graph of the thermal events of the heart gives more definite information as to what is occurring between the periods of heat production, these latter are more sharply separated and more easily read. It thus gives a completer picture of the thermal oscillations for the whole of a muscle action; this offers, I believe, a firmer basis for the comparison I have just made with the known chemical transformations.

SUMMARY AND CONCLUSIONS

1. Two points in the present investigation confirm the results of my earlier studies:

a. The rise of temperature associated with systole, and therefore with the act of muscle contraction, occurs subsequent to and not before, the beginning of rise in muscle tension.

b. The total algebraic sum of heat exchanges for the whole of a cardiac cycle is positive and its magnitude is of the order of 0.0011 small calorie per gram of muscle and per beat. This number is the same as the heat equivalent demanded by the quantity of dextrose utilized by mammalian hearts as may be computed from the results determined experimentally by several workers in widely separated laboratories. Conversely the heat observed experimentally in the present investigation is equal to an amount of dextrose equivalent to 2.96×10^{-7} gram, per gram muscle and per beat,— the number identical with that that may be computed on the basis of the metabolism experiments on mammalian hearts.

2. The new points brought out by the present investigation are as follows:

a. If one observes the muscle temperature of terrapin hearts beating at sufficiently slow rates there appear oscillations of temperature that are characteristic for the various mechanical phases of the cardiac cycle. These osciallations fall into six distinct phases. The series for a single heart beat as traced in the graphic record is termed a *thermocardiogram.*

b. While the term thermocardiogram implies that the oscillations are only peculiar to heart muscle, theoretical considerations which appear in the text indicate that further experimentation will show that the same oscillations will be found also to accompany the single twitch of skeletal muscle. The term thermocardiogram, therefore, should be considered a special term in its connotation, to be finally included in the more general term, thermomyogram.

c. The durations of the phases of the thermocardiogram as well as the temperature changes and heat values they represent have been measured and calculated, and finally compared with the successive mechanical events of the cardiac cycle.

d. The heat values of the thermocardiographic phases have further been compared with the heats of reaction as demanded by the chemical events now known to occur during the activity and recovery of frog skeletal muscle in long-continued experiments and as determined and outlined by Meyerhof.

e. From these comparisons it is shown that the rise of temperature during systole (rise of muscle tension) is more than is demanded by the sums of the heats of the anoxidative reactions. The excess of heat has been ascribed to, and has been shown in large part to be accounted for by, the thermoelastic effect of the isometrically contracting muscle.

f. During diastole a remarkable fall of temperature occurs in the thermocardiogram that can be accounted for only in small part by the reverse thermoelastic effect just alluded to. After considering the probable fall of temperature due to the nature of the thermopile used and the duration of the phase, and the possible cooling due to experimental error, it is concluded that about three-fourths of the temperature fall in this phase must still be due to endothermic processes going on within the muscle whose nature is still undetermined.

g. The post-diastolic phases of the thermocardiogram are taken to represent the phenomenon known in muscle physiology as the period of recovery. The sum of the two post-diastolic rises of temperature agree very nearly when put in terms of heat with the proportional amount demanded by the oxidation of 1/5 of the dextrose taking part in the muscle cycle (excitation to fatigue and recovery) as found experimentally by Meyerhof. The heat causing these two rises in the thermocardiogram is explained therefore not only as being due wholly to carbohydrate oxidation, but as representing practically all the heat produced by such oxidation during the whole of the cardiac cycle.

h. The rise due to this oxidative heat occurs, as stated, in two distinct phases. The stasis or fall separating them is explained as a period during which lactic acid is built up into dextrose, an endothermic process, but further one that removes lactic acid from the seat of the oxidative reaction.

i. Upon completion or retardation of the snythetic action just mentioned the oxidation of lactic acid undergoes acceleration again increasing the velocity of heat production, and giving further rise in the thermocardiogram, the "third rise." This oxidative reaction finally reaches its end-point or equilibrium, when another stasis or fall appears in the thermocardiogram. It is suggested that the final stage in the resynthesis of carbohydrate takes place during this stasis,—the condensation of dextrose into glycogen, a synthesis during recovery demonstrated by Meyerhof.

j. In very slowly beating hearts a period of resting metabolism with no temperature oscillation now occurs in the thermocardiograms, a

period prolonged without interruption until the next systole comes along, marking the end of the one and the beginning of the next cardiac cycle and its accompanying thermocardiogram.

k. It is therefore concluded that the oscillations of temperature in the terrapin's thermocardiogram for the most part faithfully follow the oscillations of the major heats of reaction, partly physical but mostly chemical, that from other lines of experimental evidence we are reasonably sure take place during the cycle of a muscle action.

l. The analysis of the thermocardiogram specifically shows that the whole history of events, as determined from the balance sheets of experiments on skeletal muscle covering hours and days of fatigue and recovery, is repeated in the short span of a single heart beat. This illuminates and makes clear at last the wonder of the heart's great regularity, its incessant vitality, the age-long mystery of its indefatigability.[3]

BIBLIOGRAPHY

(1) SNYDER: This Journal, 1917, xliv, 421.
(2) SNYDER: Ibid., 1918, xlvii, 156.
(3) SPAETH: Ibid., 1916, xli, 597.
(4) BÜRKER: Tigerstedt's Handb. d. physiol. Methodik, 1908, Bd. ii, Erste Häfte, 3te Abteilung, S. 1–86.
(5) HILL: Ergebn. d. Physiol., 1916, 15ter Jahrgang, 444.
(6) BÜRKER: Arch. f. d. gesammt. Physiol., 1919, clxxiv, 293.
(7) STEINER: Arch. f. d. gesammt. Physiol., 1875, xi, 204.
(8) See BÜRKER (4), pp. 52–53.
(9) ENGELMANN: Königl. preussische Akad. d. Wissensch., 1906, xxxix, 694. GOTSCHLICH: Arch. f. d. gesammt. Physiol., 1893, liv, 109.
(10) HILL AND HARTREE: Phil. Trans. Roy. Soc. of London, B, 1920, ccx, 153.
(11) ROHDE: Arch. f. exper. Path. u. Pharm., 1912, lxviii, 491.
(12) SNYDER: This Journal, 1909, xxv, p. xxvii.
(13) FLETCHER AND HOPKINS: Journ. Physiol., 1906, xxxv, 247.
(14) MEYERHOF: Arch. f. d. gesammt. Physiol., (I) 1920, clxxxii, 233; (II), 285; (III), clxxxv, 11: (IV), 1921, clxxxviii, 114.
(15) GARREY: Journ. Gen. Physiol., 1920, iii, 41, 49.
(16) JENSEN: Zeitschr. f. physiol. Chemie, 1902, xxxv, 514, 529.
(17) MACLEOD: This Journal, 1909, xxiii, 178.
(18) MURLIN AND SWEET: Journ. Biol. Chem., 1916, xxviii, 261.
(19) LANGFELDT: Ibid., 1921, xlvi, 381, 391, 403.
(20) HILL AND HARTREE: Journ. Physiol., 1920, liv, 84.

[3] After this work was experimentally complete Meyerhof's latest paper (14, IV) came to hand in which I find this thought expressed, albeit in the form of a prophecy.

AN EXPLANATION FOR THE INCREASE IN OXIDATION BROUGHT ABOUT BY MUSCULAR WORK

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Lavoisier was the first to show that muscular work increases oxidation in the body. Many investigators (1) since his time have worked on different phases of oxidation, and it is now known that the energy for mechanical work is derived from the oxidation of sugar and fat, principally from sugar, and that ordinarily the protein metabolism is not affected by mechanical work.

The question that has interested us is the immediate cause of this increase in the oxidative processes. Hill and his co-workers (2) have shown that the oxidations take place during the phase of relaxation, but the material oxidized and the mechanism controlling the oxidative processes remain uncertain. Lactic acid is formed during the chemical change that precedes the contraction and it plays an important rôle probably in the physical act of shortening. It seems probable also that the oxidative changes during relaxation which yield the energy to the muscle affect this substance since in the absence of oxygen muscular contractions and rigor mortis are followed by an accumulation of lactic acid (3).

Since we (4) had found that whatever increases oxidation in the body, the ingestion of food, cold baths, exposure to low temperatures, etc., produces an increase in catalase, an enzyme possessing the property of liberating oxygen from hydrogen peroxide, and that whatever decreases oxidation, narcotics for example, produces a decrease in catalase, we naturally turned to this enzyme in seeking an explanation of the increase in oxidation brought about by physical work.

The animals used were the ordinary domestic rabbits. These were given moderately severe exercise by running. The animals were placed in a treadwheel that was turned slowly by hand for 5 minutes, thus forcing the rabbit to run.[1]

[1] We desire to express our thanks to John Gere for exercising the animals.

Catalase determinations of the blood of the jugular vein were made immediately before and after the exercise. The determinations were made by adding 1 cc. of the blood diluted 1 to 3 with physiological saline to 150 cc. of neutral hydrogen peroxide in a bottle, and the amount of oxygen liberated in 10 minutes was taken as a measure of

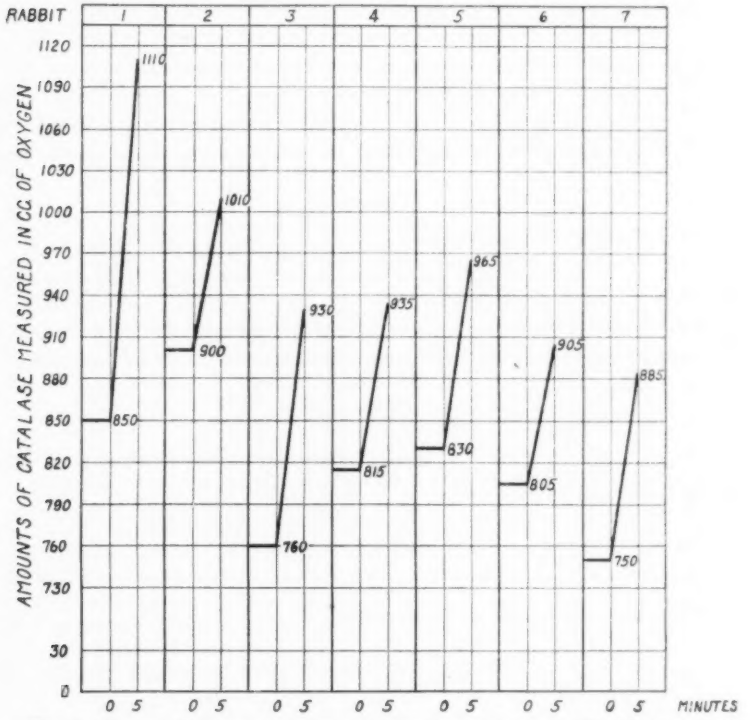

Fig. 1. Curves showing the increase in the blood catalase of rabbits brought about by 5 minutes of moderately severe exercise.

the catalase content of the blood. The material was shaken in a shaking machine during the determinations. The results of the determinations are shown in figures 1 and 2.

It may be seen in figure 1 that 1 cc. of the diluted blood of rabbit 1 liberated 850 cc. of oxygen from hydrogen peroxide immediately before the 5 minutes of exercise was begun, and 1110 cc. of oxygen immedi-

ately after the exercise. Similarly it may be seen in the chart that exercise increased the blood catalase of rabbits 2, 3, 4, 5, 6 and 7. It should be mentioned in this connection that at least twenty rabbits

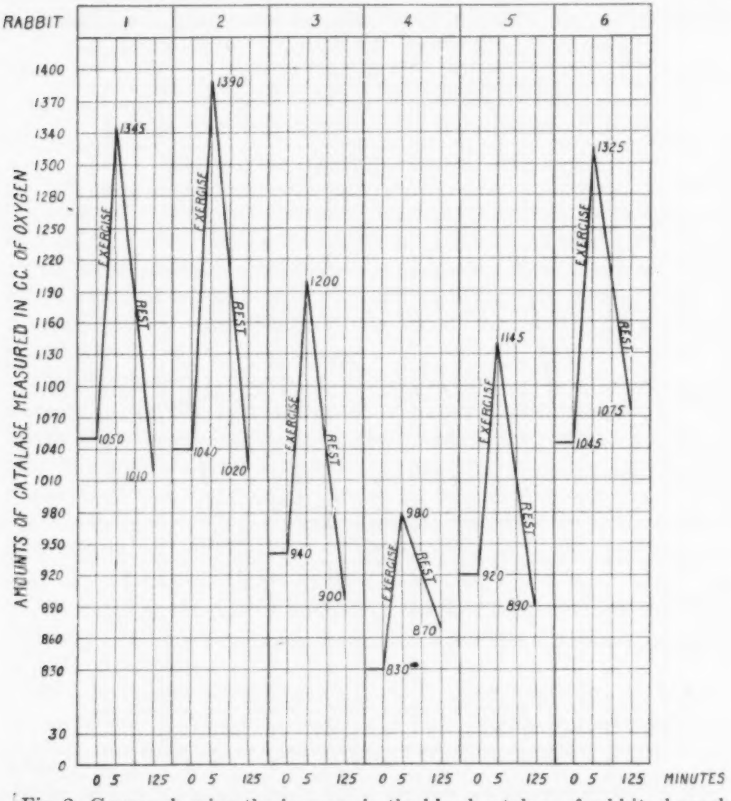

Fig. 2. Curves showing the increase in the blood catalase of rabbits brought about by 5 minutes of moderately severe exercise and the decrease in catalase with subsequent rest.

were made use of in these experiments, and all of them without an exception showed a marked increase in the blood catalase after exercise.

In figure 2 is shown the effect of exercise and subsequent rest on the blood catalase of rabbits. It may be seen that 1 cc. of the diluted blood of rabbit 1 liberated 1050 cc. of oxygen in 10 minutes from hydrogen

peroxide immediately before the exercise and 1345 cc. immediately after the 5 minutes of exercise, and that after 2 hours rest 1 cc. of the blood liberated 1010 cc. of oxygen, that is, the blood catalase had returned to normal, in fact, a little below normal. By examining the curves constructed from data obtained from rabbits 2, 3, 4, 5 and 6 it is evident that moderately severe exercise for 5 minutes increased the blood catalase, and at the end of 2 hours' rest it had returned to normal, and in some cases to a little less than normal.

It may be noticed that the catalase content of the blood of the rabbits used for data in figure 1 was in general lower than that of those for figure 2, in keeping with the fact that the rabbits for figure 1 were less vigorous and had been kept in smaller quarters than the others.

SUMMARY

Moderately severe muscular exercise increases the blood catalase, and with subsequent rest this returns to normal.

The increase in oxidation brought about by muscular work is attributed to the increase in catalase, and the decrease in oxidation with subsequent rest is attributed to the diminution in this enzyme.

BIBLIOGRAPHY

(1) Pettenkofer and Voit: Zeitschr. f. Biol., 1866, ii, 537.
Fick and Wislicenus: Myothermische Untersuchungen, 1889.
Benedict and Cathcart: Muscular work, Carnegie Inst., 1914, Publ. 187.
Fries: Biochem. Zeitschr., 1911, xxxv, 368.
Spiro: Zeitschr. f. physiol. Chem., 1877, i, 111.
(2) Hill: Journ. Physiol., 1913, xlvi, 28.
Hill and Haskins: Ibid., 1920, lxxxiv, 54.
(3) Fletcher and Hopkins: Croonian Lecture, Proc. Royal Soc. London, 1917, B lxxxix, 444.
(4) Burge: This Journal, 1916, xlvii, 351; 1921, lvi, 408.

EFFECT OF ETHYL ALCOHOL ON TADPOLES

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The following experiments on the effect of alcohol on tadpoles were begun in a haphazard way, with specimens which hatched from eggs collected for class use in the spring of 1920. A large mass of frogs' eggs (sp. ?) was brought to the laboratory on April 17. As soon as the eggs had hatched, approximately 25 tadpoles were put into each of five tumblers containing 150 cc. of tap-water and a liberal amount of Elodea, an aquatic plant. To each of two of the cultures there was added 10 cc. of 10 per cent alcohol and to each of two others 10 cc. of 5 per cent alcohol, making approximately $\frac{2}{3}$ and $\frac{1}{3}$ per cent alcohol respectively. All five cultures, four containing alcohol and one not, were placed side by side on a table in strong diffuse light. The solution in all of the cultures was renewed every morning.

In the course of three weeks it could be readily seen that the tadpoles in the cultures containing alcohol were considerably larger than those in the control culture. The mortality was, however, so high in all of the cultures that there were only a few specimens left at this time and there was, consequently, considerable doubt as to the significance of the results. The experiment was therefore repeated in the spring of 1921.

A large number of frogs' eggs, all deposited by one female (sp. ?), was brought to the laboratory on March 20. Two days later there were numerous tadpoles. These were taken at random, March 22, and put into cultures in 6 battery jars, 5 approximately 12 cm. in diameter and 1 approximately 15 cm. in diameter. Two of the small jars contained each 700 cc. of $\frac{2}{3}$ per cent alcohol, one with plants (Elodea and Nitella) the other without. Two contained $\frac{1}{3}$ per cent alcohol, one 700 cc. with plants, the other 350 cc. without plants. The remaining small jar contained 350 cc. tap-water without plants; and the large jar contained 1000 cc. tap-water with plants. The number of specimens put into the various jars is indicated in table 1. Tap-water was used in preparing the solutions of alcohol in all of the experiments. In addi-

tion to Elodea and Nitella there were also present some diatoms and other unicellular forms. The tadpoles fed freely on all of these, the latter as well as the former. No food was added to any of the cultures except the plants and alcohol as indicated above.

The jars were all kept side by side in strong indirect sunlight and the solution in each one was renewed every morning.

Whenever the tadpoles were put into a fresh solution of alcohol they became extremely active and swam about violently for a few minutes,

TABLE 1

Effect of alcohol on length of life in tadpoles

DATE	NUMBER OF TADPOLES ALIVE					
	In 700 cc. ¼ per cent alcohol with plants	In 700 cc. ¼ per cent alcohol, no plants	In 700 cc. ½ per cent alcohol with plants	In 350 cc. ½ per cent alcohol, no plants	In 1000 cc. tap-water with plants	In 350 cc. tap-water, no plants
March 22	50	15	50	15	100+	25
March 26	25*	15*	50	15	100+	25
April 4	25**	?**	50	?	?	13
April 9	25	?	50‡	?	?§	0
April 22	25	4	35	4	? Many dead	
May 6	8	0	13	3	5	
May 8	2		?	3	?	
May 23	0		7	0	4	

* Alcohol reduced to ⅛ per cent.

** Alcohol increased to ⅜ per cent.

‡ Fifteen tadpoles taken out at random, leaving 35.

§ Fifty-two tadpoles taken out at random, some April 9, the rest earlier.

From 3/24 to 4/24 the tadpoles were in alcohol from morning until evening, about 10 hours daily; from 4/24 to 5/12 continuously, from 5/12 on about 10 hours daily. The alcohol and the water were renewed in all of the cultures every morning.

then they became very sluggish but the movements continued to be well coördinated and there was no indication of intoxication. On the whole, those in the tap-water were, however, more active than those in alcohol and they responded more vigorously to stimulation.

Further results obtained are presented in tables 1 and 2. By referring to table 1 it will be seen that the tadpoles which were subjected to alcohol lived longer than those which were not, but that those in the weaker solutions lived longer than those in the stronger. This is particularly clear in the solutions with no plants. In these the table shows

that while all of the tadpoles in the culture without alcohol were dead in 18 days, in that containing $\frac{2}{3}$ per cent alcohol 4 were still alive after 31 days and in that containing $\frac{1}{3}$ per cent 3 were alive after 47 days. The table also indicates that the rate of mortality in the cultures with plants was lowest in the one containing $\frac{1}{3}$ per cent alcohol.

After the tadpoles had been in the solutions a few weeks it was very evident that those in the alcohol, especially $\frac{2}{3}$ per cent, were larger than those in water. This is substantiated by the results of measurements presented in table 2.

This table shows that on May 6, 45 days after the experiment was started, the tadpoles which had been in $\frac{2}{3}$ per cent alcohol averaged 21 mm. in length and those in $\frac{1}{3}$ per cent 18.55 mm., while those in tap-

TABLE 2

Effect of alcohol on the rate of growth in tadpoles

DATE	NUMBER OF INDIVIDUALS	CULTURE SOLUTION	AVERAGE TOTAL LENGTH	AVERAGE LENGTH OF BODY	AVERAGE WIDTH OF BODY
			mm.	*mm.*	*mm.*
May 6	8	$\frac{2}{3}$ per cent alcohol with plants	21.00	7.60	4.55
May 6	13	$\frac{1}{3}$ per cent alcohol with plants	18.55	6.86	4.21
May 6	3	$\frac{1}{3}$ per cent alcohol without plants	19.30	7.00	4.25
May 6	5	Tap-water with plants	17.00	6.40	3.98
May 23	7	$\frac{1}{3}$ per cent alcohol with plants	19.24	7.14	4.32
May 23	4	Tap-water with plants	16.31	6.44	3.87

All of the experiments were started on March 22.
For details regarding methods see text and table 1.

water averaged only 17 mm., and that on May 23 those in $\frac{1}{3}$ per cent alcohol averaged 19.24 mm. in length, while those in tap-water averaged only 16.31 mm. It shows also that similar differences obtained in the other dimensions considered.

The variations in the dimensions, on the other hand, were essentially the same under the various conditions; for example, the tadpoles in $\frac{2}{3}$ per cent alcohol on May 6 varied in length from 18 to 23 mm., those in $\frac{1}{3}$ per cent from 15.5 to 21 mm., and those in tap water from 16 to 18 mm. On May 23 those in $\frac{1}{3}$ per cent alcohol varied from 17 to 21.25 mm., and those in tap-water from 14 to 18 mm.

Figure 1 gives a good idea of the difference in the size of the tadpoles in two of the cultures at the close of the experiment, one contain-

ing alcohol, the other not. It can be clearly seen from this figure, which is a reproduction of a photograph, that at this time the tadpoles in the culture containing alcohol were considerably larger than those in the culture without alcohol.

The evidence presented seems to show conclusively that a weak solution of alcohol causes an increase in the rate of growth in tadpoles and tends to reduce the rate of mortality. As to how these effects are

Fig. 1. Photograph of tadpoles showing effect of alcohol on rate of growth. *A*, in ½ per cent alcohol, from March 22 to May 23; *B*, in tap-water, from March 22 to May 23.

produced is not clear. The alcohol, as previously stated, produced a reduction in activity. This might account for the increase in length of life, but it does not account for the increase in the rate of growth. It would consequently appear to be more reasonable to assume that the alcohol served as food, probably primarily by being directly oxidized in the body, thus furnishing energy and conserving other food material.

We hope to continue these experiments during the coming season.

POULTRY FLESHING INVESTIGATIONS

The Utilization of Soy Bean and Corn Proteins as Affected by Suitable Mineral Supplements

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One of the most vital needs of the poultry industry today is a solution of the more important problems in connection with the feeding of poultry. There is a dearth of positive information relative to the more fundamental principles of poultry nutrition, the need of which is keenly felt by every progressive poultry feeder.

It is evident that if poultry and eggs are to be produced more efficiently, more effective and less expensive feed combinations must be developed. This can be accomplished only by systematic investigation of poultry nutrition. Some of the more important problems which invite attention are: *1*, Comparative value of animal and vegetable proteins; *2*, efficient combinations of proteins for rapid growth and egg production; *3*, the amount and the state of division of crude fiber in rations for the best results; *4*, vitamine requirements of poultry; *5*, supplements for rations deficient in essential mineral elements.

The importance of the quality and quantity of the mineral elements in foods is generally recognized. Ample mineral elements are essential to the well-being of all animals. The likelihood of a deficiency of inorganic matter becoming a limiting factor in growth is quite great in the case of the rapidly growing animal which receives a ration consisting largely of concentrates with a small proportion of roughage. This is particularly true of swine and chickens. The chicken is physiologically unable to utilize crude fiber and is not so constituted as to be able to ingest a large amount of roughage which contains a larger porportion of inorganic matter than the corresponding concentrates. For this reason special attention should be given to the mineral content of rations for chickens. The other farm animals excepting swine, as usually fed, receive a larger proportion of roughage, the mineral content of which usually serves to meet their requirements.

Hart (1), in his animal nutrition studies, in which he employed rations limited to corn or wheat grains and their products, found consistent agreement with rats and swine, while chickens behaved entirely differently. He concluded that the mineral requirements at least and possibly the requirements for the other nutritive factors were not the same for chickens as they were for mammals.

The importance of inorganic matter in rations for growing chickens and the deficiency of grains and their by-products in this respect has been emphasized by Wheeler (2) who found by numerous tests that the addition of animal feed to grain rations always gave better results than grains alone. However, when the grain rations were supplemented by bone ash they proved about equal to the ones containing the animal meal. He concludes that it is the deficiency of essential minerals in the grain rations that makes them inferior to those containing the animal material rather than a difference in the quality of the proteins.

One of the vegetable protein concentrates which gives promise as a valuable constituent of poultry feeds is soy bean meal. Osborne and Mendel (3) and Daniels and Nichols (4) have found that the proteins of soy beans are of high quality. Corroborative evidence is furnished by Philips et al. (5) who showed that soy bean meal, when supplemented by a suitable salt mixture, was superior to meat scraps in supplying the protein needs of chicks grown in confinement.

Although but a limited amount of research has been performed dealing with the nutrition of chickens as compared with other animals such as rats, guinea pigs, swine and cattle, that which has been done shows conclusively that the results obtained with other animals cannot be translated to apply directly to chickens. The valuable and extensive work accomplished with other animals may be used for the suggestions they afford, but conclusive data applying to chickens can only be secured by research conducted on these animals. The purpose of the present investigation is to ascertain the value of soy bean meal as a consitutent of poultry rations with a view to the practical application of the findings to the needs of the poultry fattening industry. The study deals with the following questions: Is soy bean meal deficient in mineral matter to such an extent as to affect its feeding value; if so, what is the nature of the deficiency, what is the most efficient way of overcoming it, and what effect does it have on the assimilation of protein and the storage of fat.

EXPERIMENTAL. The experiments were conducted with birds confined in metal batteries similar to the usual commercial feeding batteries except

they were partitioned into individual compartments 9 inches × 18 inches. Barred Pymouth Rock cockerels from standard bred stock were carefully selected for uniformity of size, quality and vigor, from large numbers of birds received by a market poultry dealer. Each bird was weighed at the beginnng of the experiment and at the end of the fourth, eighth and fourteenth days. The birds received their feed twice daily (8 a.m. and 4 p.m.) in form of a batter in individual cups so constructed as to prevent all possible waste of feed thus making it possible to secure accurate daily individual feed records. In case a bird died or proved unable to function normally it could be eliminated without affecting the accuracy of the feed records of the other birds receiving the same ration. No water was given the birds except that which was added to the dry feed mixture to make it of such consistency that it would pour from one container to another.

A ration consisting solely of corn meal and buttermilk (40:60) had been used as a control in numerous feeding experiments previously conducted by this laboratory. A survey of these experiments shows that broilers and springs fed on this ration for 14 days gain an average of 30 per cent in weight. Three rations were used in the present investigation. These consisted of a basal ration,—corn meal, soy bean meal and water; the basal ration plus various salt mixtures; and the corn meal and buttermilk ration, the latter being used only in the first series of experiments as a control. The composition of these rations will be found in table 1. The mineral content of the basal and basal-plus-salts rations is given in table 2. The ash of the buttermilk ration was not examined. Various salt mixtures used in the investigation are listed in table 3.

As an arbitrary numerical measure of the relative merits of the rations, the values of the rations were expressed in terms of one of the rations using figures based upon the grams of feed per gram of gain. These values were obtained by dividing the figures for the grams of feed per gram of gain of all the rations used in the experiment by the figure for the grams of feed per gram of gain of the particular ration used as a base and multiplying by 100. The rations used as a base will, of course, receive the value of 100, those superior and inferior to it will be represented by numbers greater and less, respectively, than 100.

At the beginning and at the end of the feeding experiment specimens representative of the unfed and of the fleshed birds were killed, carefully bled, dry picked, and chilled for 24 hours in a room at 33°F. After weighing (chilled weight), they were dissected so as to separate the

TABLE 1

Composition of rations

RATION		METHOD OF STATING ANALYSIS	MOISTURE	TOTAL SOLIDS	PROTEIN	FAT	NITROGEN FREE EXTRACT	FIBER	ASH
Name	Ingredients								
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Corn meal and buttermilk	Corn meal 40% Buttermilk 60%	In per cent of wet mash	60.62	39.38	5.89	2.13	29.31	1.09	0.96
		In per cent of moisture-free material		100.00	14.96	5.41	74.42	2.76	2.44
Basal	Corn meal 32.4% Soy bean meal 7.6% Water 60.0%	In per cent of wet mash	64.09	35.91	6.38	2.08	25.38	1.21	0.86
		In per cent of moisture-free material		100.00	17.76	5.80	70.68	3.36	2.40
Basal plus salts	Corn meal 31.2% Soy bean meal 7.6% Salt mixture no. 4 1.2% Water 60.0%	In per cent of wet mash	63.96	36.04	6.26	2.03	24.54	1.17	2.04
		In per cent of moisture-free material		100.00	17.25	5.63	68.15	3.25	5.58

edible from the inedible part. A composite sample of the meat, skin and edible organs was prepared for analysis by grinding five or six times to insure a thorough mixture. The official methods of the Association of Official Agricultural Chemists (6) were used for determinations of total solids, crude fat and nitrogen, the latter being determined by the Kjeldahl-Gunning method.

TABLE 2

Mineral content of rations

RATION	MINERAL CONSTITUENTS*							
	P	Cl	Ca	Mg	Na	K	S	Fe
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Basal	0.337	0.033	0.100	0.178	0.156	0.565	0.217	0.008
Basal-plus-salts no. 4	0.717	0.386	1.144	0.252	0.423	0.554	0.212	0.008

* P, Cl and Ca were determined in a composite sample of each ration by official methods of the Association of Official Agricultural Chemists(10). The proportions of the other elements were calculated from analyses of corn meal and soy bean meal reported by Kaupp (11) and from an anlaysis of the water supplied by the Indianapolis Water Company.

TABLE 3

Salt mixtures

INGREDIENTS	SALT MIXTURE				
	No. 1	No. 2	No. 3	No. 4	No. 5
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Calcium carbonate	14.0	16.67	80.00	20.00	100.00
Sodium chloride	15.0	17.86	20.00	20.00	
Bone ash	45.0	53.57		60.00	
Di-potassium phosphate	10.0	11.90			
Calcium lactate	10.0				
Magnesium sulphate	3.0				
Sulphur	2.0				
Ferrous sulphate	1.0				

In one of the experiments comparative data on nitrogen retention were obtained by determination of the nitrogen content of the food and feces during a feeding period of 4 days. A preliminary feeding interval of 5 days preceded the experimental period proper, which served the following purposes: to accustom the birds to their confinement; to obtain data on the average voluntary consumption of feed; to eliminate from

the alimentary tract any residues of previous feeds; and to give an opportunity to select birds functioning normally. The excrement was collected for 4 days upon galvanized iron pans placed under the individual feeding compartments. The samples were removed from the pans at morning and night just before feeding, placed in 95 per cent alcohol slightly acidified with acetic acid, and dried on a water bath.[1] The samples were finely ground, weighed and analyzed for total nitrogen by the Kjeldahl-Gunning-Arnold method.

DISCUSSION OF RESULTS. Upon examination of the data in table 4 it will be observed that the salt mixture no. 1 had a marked effect in increasing the efficiency of the basal ration. The gains obtained with the corn meal and buttermilk ration and the modified basal ration compare favorably with each other, being 29.52 per cent and 28.62 per cent, respectively. The soy bean meal plus salts was but slightly

TABLE 4

Corn meal supplemented by buttermilk, soy bean meal, and soy bean meal plus salt mixture no. 1

RATION	NUMBER OF EXPERIMENTS	AVERAGE INITIAL WEIGHT PER BIRD	AVERAGE GAIN IN 12 DAYS		FEED PER GRAM OF GAIN	RELATIVE VALUE OF RATIONS—BASAL-PLUS-SALTS, NO. 1 = 100
		grams	*grams*	*per cent*	*grams*	*per cent*
Corn meal and buttermilk.....	3	908	268	29.52	3.41	95.31
Basal.....	3	897	168	18.73	4.74	68.57
Basal-plus-salts no. 1.....	3	891	255	28.62	3.25	100.00

inferior to the buttermilk as a supplement to corn meal whereas the soy bean meal alone proved to be distinctly inferior in this capacity. The basal ration is clearly deficient in salts.

Since the salt mixture no. 1 proved effective in the correction of the mineral deficiency of the basal ration it seemed desirable to determine if a more simple mixture would prove of equal value. In other words, what are the really essential elements or compounds responsible for the benefit derived from the salt mixture no. 1? In an endeavor to gain information on this question a study was made of the effect upon the growth of chickens produced by additions of each of five different salt mixtures (table 3) to the basal ration. The added mineral matter

[1] Poda (7) applied this method successfully to human feces. Brown (8) and Bartlett (9) report good results from the use of this method for the preservation of chicken excrement.

constituted 3 per cent of the dry feed mixture in each case. As shown in table 5, calcium carbonate and sodium chloride in the proportion of 4 to 1 were considerably more efficient than calcium carbonate alone. The latter appeared to be detrimental rather than helpful. The best results were obtained with salt mixtures nos. 2 and 4, which contained a high proportion of bone ash. It is evident that salt mixture no. 4 consisting of bone ash, calcium carbonate and sodium chloride (60:20:20), is the simplest of the mixtures which adequately corrected the mineral deficiency of the basal ration. The value of sodium chloride and of the minerals found in bone ash as supplements for the corn meal and soy bean meal ration is shown in this experiment.

TABLE 5

Corn meal and soy bean meal (basal ration) supplemented by different salt mixtures

RATION	NUMBER OF EXPERIMENTS	AVERAGE INITIAL WEIGHT PER BIRD	AVERAGE GAIN IN 14 DAYS		FEED PER GRAM OF GAIN	RELATIVE VALUE OF RATIONS—BASAL-PLUS-SALTS NO. 1 = 100
		grams	*grams*	*per cent*	*grams*	*per cent*
Basal	3	1136	257	22.62	5.05	79.60
Basal plus 3 per cent salts no. 1	3	1136	332	29.23	4.02	100.00
Basal plus 3 per cent salts no. 2	3	1117	336	30.08	3.77	106.63
Basal plus 3 per cent salts no. 3	3	1144	274	23.95	4.45	90.34
Basal plus 3 per cent salts no. 4	3	1136	329	28.96	3.96	101.52
Basal plus 3 per cent salts no. 5	3	1118	200	17.89	6.09	66.01

While a 3 per cent addition of salt mixture no. 4 proved effective, there was no certainty as to whether this was the optimum amount. There seems to be no definite information on this point and it is customary for investigators to add arbitrarily a certain amount of salt mixture to a ration, the object being to make sure the amount added fully supplements the deficiency to be corrected. The amount of the salt mixture added to a ration by different investigators varies from 2 to 10 per cent of the ration. In order to determine more definitely the optimum amount of the salt mixture no. 4 required for the basal ration, various proportions (2 per cent, 3 per cent, 5 per cent and 7.5 per cent) of the salt mixture were added. The data in table 5 do not clearly indicate the optimum amount to be added, but it will be seen that the smaller amounts seem to be slightly more effective, though no positive disadvantage resulted from the use of the larger amounts.

In the course of the season's work (July to December, 1920) nine different experiments were conducted, in which means were afforded for comparing the values of the basal ration and the same ration plus 3 per cent of salt mixtures nos. 1 and 4. The growth curves for these

TABLE 6

Corn meal and soy bean meal (basal ration) supplemented by different proportions of salt mixture no. 4

RATION	NUMBER OF EXPERIMENTS	AVERAGE INITIAL WEIGHT PER BIRD	AVERAGE GAIN IN 14 DAYS		FEED PER GRAM OF GRAIN	RELATIVE VALUE OF RATIONS—BASAL PLUS 3 PER CENT SALTS NO. 4 = 100
		grams	*grams*	*per cent*	*grams*	*per cent*
Basal	3	1146	218	19.02	5.09	71.32
Basal plus 2 per cent salts no. 4	3	1201	335	27.89	3.50	103.71
Basal plus 3 per cent salts no. 4	3	1200	310	25.83	3.63	100.00
Basal plus 5 per cent salts no. 4	3	1233	297	24.09	4.01	90.52
Basal plus 7½ per cent salts no. 4	3	1258	307	24.40	4.04	89.85

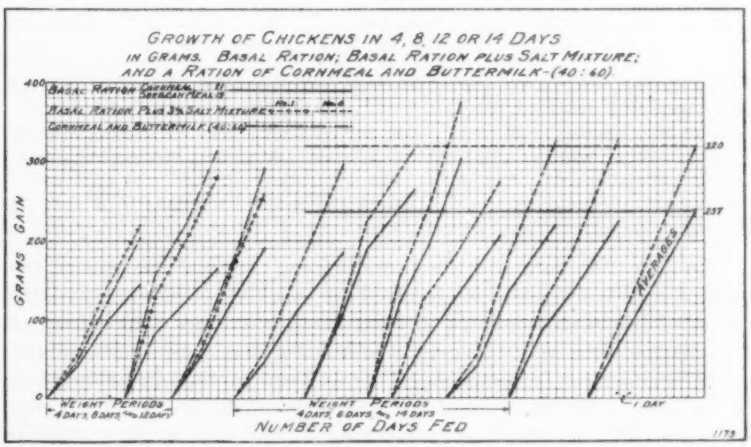

Chart 1

experiments are represented in the chart above. Each experiment was continued for 14 days except the first three, which ended after 12 days. In every case the corn meal and soy bean meal ration supplemented by the salt mixture proved distinctly superior to the same ration

(basal) without the salt mixture, the average increased gain resulting from the use of the salts being 38.86 per cent. Since the voluntary feed intake of the birds receiving the two rations was practically the same, any constant difference in the results secured was attributed to the salt mixture. It appears, therefore, that the benefit derived from the salts was due to better metabolism rather than to the effect of a tonic or appetizer. The average gain made by the birds receiving the basal ration plus the salt mixture for the nine different lots was 28.05 per cent. This compares favorably with the average gain of 30 per cent from the corn meal and buttermilk ration.

TABLE 7

Amount and composition of edible portion of birds

TIME OF EXAMINATION	NUMBER OF EXPERIMENTS	NUMBER OF BIRDS DISSECTED	AVERAGE FINAL LIVE WEIGHT OF BIRDS	AVERAGE WEIGHT OF CHILLED BIRDS	AVERAGE WEIGHT OF EDIBLE PORTION OF BIRDS			COMPOSITION OF EDIBLE PORTION OF BIRDS		
					Total	In per cent of chilled weight	In per cent of final live weight	Crude fat	Protein	Water
			grams	*grams*	*grams*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Before feeding.....	12	72	895	797.0	434.4	54.50	48.54	7.91	20.28	71.12
After receiving basal ration for 14 days...	2	12	1055	949.5	521.2	54.89	49.40	20.18	18.03	61.57
After receiving basal ration plus 3 per cent salts no. 4 for 14 days	4	16	1206	1064.8	603.3	56.66	50.02	14.01	19.11	66.30

The dissection data and the corresponding chemical analyses of the control birds and those which received the basal ration with and without the salt mixture are presented in table 7. The average composition of the control birds was taken as representing the composition of the experimental birds before feeding. The 72 control birds were found to be quite uniform in composition and any consistent change during feeding would, therefore, be revealed by the analysis of the fed birds. Some idea of the general character of the growth produced by the two rations can be gained in this manner. The composition of the edible part of the birds fed the basal ration plus the salt mixture was markedly different from that of those receiving the basal ration. The latter contained a larger proportion of fat and a smaller porportion of protein and water.

In order to afford an actual comparison of the amounts of fat, protein and water added as growth by the chickens, the average weights of these constituents on the edible parts of the birds before and after feeding were calculated from the data of the average composition of the birds. The differences between the weights of fat, protein and water in the edible portion of the birds before and after feeding are represented as the gains of these constituents as shown in table 8. The birds fed the basal ration gained 197 grams per bird or 111 grams edible parts, whereas those fed this ration plus the salt mixture gained 265 grams total or 170 grams edible. The total edible gain produced with the basal ration was composed of approximately 66.1 per cent fat, 8.9 per cent protein and 25.0 per cent water; whereas the edible growth made

TABLE 8

*Amounts of fat, protein and water added to edible portion during feeding period of fourteen days**

RATION	AVERAGE GAIN IN CHILLED WEIGHT OF BIRDS	AVERAGE GAIN IN WEIGHT OF EDIBLE PORTION OF BIRDS	COMPOSITION OF ADDED EDIBLE PORTION					
			Average amount per bird			In per cent of fat plus protein plus water		
			Fat	Protein	Water	Fat	Protein	Water
	grams	*grams*	*grams*	*grams*	*grams*	*per cent*	*per cent*	*per cent*
Basal	197	111	74	10	28	66.1	8.9	25.0
Basal plus 3 per cent salts no. 4	265	170	56	26	87	33.1	15.4	51.5

* These figures are approximations based on calculations, using analyses of representative samples of birds dissected.

with the basal ration plus the salts consisted of 33.1 per cent fat, 15.4 per cent protein and 51.5 per cent water. This seems to indicate that the mineral deficiency of the basal ration interfered with the ability of the birds to form protein tissue, and resulted in an increased storage of fat. The ration containing the salt mixture produced a greater edible growth which contained about 75 per cent more protein.

The effect of the use of the salt mixture no. 4 on the nitrogen retention of the birds is shown in table 9. On an average daily intake of 1.643 grams of nitrogen per bird, those fed the basal ration retained 0.626 gram or 38.10 per cent. With a similar nitrogen intake (1.626 grams) the birds receiving the same ration plus the salt mixture retained an average of 0.790 gram or 48.53 per cent, which is 27.38 per cent increased

retention over that of the birds fed the basal ration. These results check those given in table 8 and strengthen the view that the salts aid in the assimilation of the protein.

TABLE 9

Nitrogen retention as affected by addition of salt mixture to basal ration

RATION	NUMBER OF EXPERIMENTS	DAYS FECES COLLECTED	FEED INTAKE (MOISTURE-FREE)	FECES (AIR-DRY)	NITROGEN			
					Consumed	Excreted	Retained	
							Total	In per cent of nitrogen consumed
			grams	*grams*	*grams*	*grams*	*grams*	*per cent*
Basal	3	4	61.3	15.2	1.643	1.017	0.626	38.10
Basal plus 3 per cent salts no. 4	4	4	60.3	17.39	1.626	0.838	0.790	48.53

CONCLUSIONS

Corn meal and soy bean meal (81:19), the basal ration, supplemented by the essential mineral elements, produced gains in broilers and springs nearly equal to that obtained by the use of a ration of corn meal and buttermilk (40:60), while the basal ration without the mineral suppplement proved distinctly inferior.

A salt mixture (no. 4) consisting of bone ash, calcium carbonate and sodium chlorid (60:20:20) was the simplest mixture which proved effective in overcoming the mineral deficiency of the basal ration.

A mixture of calcium carbonate and sodium chloride (80:20) was but slightly inferior to salt mixture no. 4, but calcium carbonate alone was entirely ineffective.

The optimum amount of the salt mixture no. 4 to be added to the basal ration appears to be approximately 2 per cent of the dry feed; however, there seems to be a considerable margin as to the amount which may be added and yet obtain about equal results.

In nine experiments of 12 birds to each ration with a similar voluntary feed intake the average increased gain resulting from the addition of the salt mixtures to the basal ration was 38.66 per cent.

It appears that the mineral deficiency of the basal ration is not such as to interfere with the storage of fat but rather accelerates its formation at the expense of protein growth.

The increase of flesh produced by the ingestion of the basal ration supplemented by the salt mixture contained 61.38 per cent more protein than that obtained by feeding the basal ration without the salt mixture. The birds receiving the supplemented ration retained 27.38 per cent more of the nitrogen ingested than did those receiving the basal ration alone.

BIBLIOGRAPHY

(1) Hart, Halpin and McCollum: Journ. Biol. Chem., 1917, xxix, 57.
(2) Wheeler: N. Y. Agric. Exper. Sta., Geneva, Bull. 171, 1899.
(3) Osborne and Mendel: Journ. Biol. Chem., 1917, xxxii, 369.
(4) Daniels and Nichols: Journ. Biol. Chem., 1917, xxxii, 91.
(5) Philips, Carr and Kennard: Journ. Agric. Research, 1920, xviii, 391.
(6) Association of Official Agricultural Chemists. Methods of Analysis. 1916, pp. 5–7, 79, 80.
(7) Poda: Zeitschr. f. physiol. Chem., 1898, xxv, 355.
(8) Brown: Bull. 56, Bureau of Animal Industry, U. S. Dept. of Agriculture.
(9) Bartlett: Maine Agric. Exper. Sta. Bull. 184.
(10) Association of Official Agricultural Chemists. Methods of Analysis. 1916, pp. 31, 32.
(11) Kaupp and Ivy: Journ. Agric. Research, 1920, xx, 143.

SOME PLANT SOURCES OF VITAMINS B AND C[1]

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In the problem of food selection, instinct is considered by many persons to be a fairly safe guide. Therefore it is often assumed that the question of vitamin deficiency in the diet needs no special consideration. This, however, is not invariably the case. In the Philippines, where fruits and vegetables abound all the year round, deficiency diseases have occurred and are occurring among the Filipinos as well as among the people living in the temperate zones. That the people who can obtain the foods which are generally considered as excellent sources of vitamins should be subject to deficiency diseases may be due to one or both of the following reasons: They are either eating more and more of artificially treated foods or are not eating the right kind. So far no systematic investigation of the vitamin content of Philippine food materials has been made, except that of Brill and Alincastre on "the possible maximum vitamin content of some Philippine vegetables" (3). In the light of our newer knowledge concerning these mysterious substances, the above work appears of no value. Therefore in a series of studies of the nutrition of the Filipinos it became highly desirable that vitamin tests be made on the fruits and vegetables which are being or may be eaten by them.

Several vegetables and fruits have already been tested for vitamin content by different investigators (27), (28), (7), (34), (9), (16), (21), (30), (31), (32), (33), (17), (18), (26), (24), (11), (4), (8), (20), (35). A thorough review of the subject shows, however, that there are still many uninvestigated, which are commonly eaten. The present work is intended as a beginning of a series of tests which will be undertaken soon. It is regretted that the distance from the Philippines has made

[1] The data in this paper are taken from the dissertation presented by the writer for the degree of Doctor of Philosophy, Yale University, 1922.

[2] Traveling Fellow of the University of the Philippines.

the selection of materials limited to what could be purchased here in the States and what were furnished by Doctor Kellogg of the Battle Creek Sanatorium, the United Fruit Co., and Mr. Ames, Director of the Botanic Garden of Harvard University in Cuba, to whom I desire to express my hearty appreciation. The present tests became dependent on the supply of materials available.

I. TEST FOR VITAMIN B

Method. In order to obtain a comparative estimate of the vitamin content of the materials under investigation a plan similar to the one used by Osborne and Mendel (32) has been adopted. The fruits and vegetables were dried in an air current at less than 85°C. The dried materials were ground finely and then made into pills of 1 gram, ½ gram, or less, according to the need of the experiments. Glucose syrup was used as adhesive.

Osborne and Mendel (31) have prepared a diet for albino rats which has been shown to be complete in every respect except that it lacks vitamin B. This mixture was slightly modified in percentage composition as follows:

Casein	18
Salt mixture[3]	4
Starch	50
Butter fat	9
Lard	19

On this food the rats slowly lose appetite and after a time decline in weight. Then if the diet is not changed or improved by the addition of substances containing vitamin B, death ensues. In most cases the animals promptly improve in appetite when vitamin B is given. But when the source of vitamin is too bulky so that it prevents the ingestion of sufficient of the standard food, the *total* intake of all essentials may become inadequate. The ability to cause the recovery of rats which have been declining in weight on a diet which is known to be complete in every respect except that it lacks vitamin B, has been taken as a proof that the material in question contains the latter.

The feeding technique was essentially like that commonly employed in this laboratory and described by Ferry (12). Protocols of body weights and food intakes are given in tables, and some of the data

[3] The salt mixture used is that described by Osborne and Mendel, Journ. Biol. Chem., 1917, xxxii, 317.

are also presented in graphic forms in charts. (Tables 1 to 10 and charts 1 to 12.)

TABLE 1

Mongo; body weight and weekly intake of standard diet

FEEDING PERIOD	ONE GRAM SUPPLEMENT							
	Rat 3 ♂		Rat 10 ♀		Rat 9 ♀		Rat 7 ♀	
	Body weight	Diet	Body weight	Diet	Body weight	Diet	Body weight	Diet
weeks	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
	150							
	168		66					
	178		83		64		70	
	194		95		94		93	
1	174	?	94	33	94	43	99	46
2	164	?	90	34	97	30	92	29
3	152x	?	85	16	90	20	82	20
4	166	46	75	16	84	16	81	16
5	178	44	64x	?	80x	12	76	16
6	190	46	85	29	98	32	76x	12
7	210	53	102	36	108	36	97	36
8	220		104	36	107	32	115	45
9			117	46	115	38	122	46
10			126	40			132	44

FEEDING PERIOD	ONE-HALF GRAM SUPPLEMENT					
	Rat 3 ♂		Rat 11 ♀		Rat 9 ♀	
	Body weight	Diet	Body weight	Diet	Body weight	Diet
weeks	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
	220					
	218		166		115	
1	202	?	153	34	122	40
2	195	?	150	29	123	40
3	184x	?	140	25	117	27
4	192	45	130x	23	100x	?
5	198	50	143	34	118	36
6	198	43	152	37	120	36
7	196y	54	154	52	132	40
8	206	56	154y	42	136	37
9	231	55	172	50		

DISCUSSIONS OF THE MATERIALS USED AND THE FEEDING EXPERIMENTS. MONGO: (*Phaseolus mongo L.*). This bean contains about

20 per cent protein (N × 6.25) (2). It is extensively used by the Filipinos and is practically the only food of the people afflicted with

TABLE 2

Togi; body weight and weekly intake of standard diet

FEEDING PERIOD	ONE GRAM SUPPLEMENT					
	Rat 1 ♀		Rat 8 ♂		Rat 2 ♀	
	Body weight	Diet	Body weight	Diet	Body weight	Diet
weeks	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
	136				134	
	144		67		144	
	150		95		154	
1	140	?	112	41	138	?
2	116x	?	103	29	102x	?
3	136	43	98x	?	136	56
4	150	49	118	35	160	62
5	152	52	135	31	162	50
6	153	36	149	42	174	46
7	156	40	164	35	180	
8	162	47	175	36		
	ONE-HALF GRAM SUPPLEMENT					
	Rat 11 ♀		Rat 13 ♂		Rat 6 ♀	
	130					
	144				74	
	156		165		98	
1	156	33	162	60	105	40
2	154	35	159	46	100	34
3	141	16	150	26	92	24
4	130	16	140	17	92	28
5	118x	15	128	?	94	27
6	130	44	122x	?	98	23
7	146	50	132	50	92	21
8	153	36	144	39	90	23
9	159	39	153	42	82x	24
10	166	38	165	46	98	41
11			177	47	120	46
12					135	39
13					148	48

wet beriberi who at the same time cannot buy milk The mongo used was bought from a Chinese grocery store at New Haven. The bean

TABLE 3

Sweet potato leaves; body weight and weekly intake of standard diet

FEEDING PERIOD	ONE GRAM SUPPLEMENT				ONE-HALF GRAM SUPPLEMENT			
	Rat 1 ♀		Rat 2 ♀		Rat 3 ♂		Rat 4 ♂	
	Body weight	Diet	Body weight	Diet	Body weight	Diet	Body weight	Diet
weeks	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
	132		126		198		186	
1	128	43	124	40	190	47	181	43
2	128	37	113	24	184	49	170	39
3	113x	15	100x	15	168	41	159	29
4	124	35	115	27	148x	15	138x	16
5	136	49	134	44	150	29	128	21
6	144	49	144	48	168	52	121	18
7	150	44	154	39	178	44	132	36
8					194	49	140	29
9							143z	37
10							159	52
11							153y	48
12							164	50
13							180	45
14							195	49

z = 0.2 gram yeast was given as daily supplement.

TABLE 4

Bilimbi; body weight and weekly intake of standard diet

FEEDING PERIOD	ONE GRAM SUPPLEMENT			
	Rat 26 ♀		Rat 6 ♀	
	Body weight	Diet	Body weight	Diet
weeks	*grams*	*grams*	*grams*	*grams*
	127		140	
1	130	37	136	31
2	132	38	130	27
3	137	41	124	27
4	130	33	122	?
5	116x	28	120x	?
6	110y	11	115d	?
7	137	52	132	41
8	160	67		

d = given one gram duhat daily instead of bilimbi.

TABLE 5

Duhat; body weight and weekly intake of standard diet

FEEDING PERIOD	ONE GRAM SUPPLEMENT			
	Rat 18 ♂		Rat 6 ♀	
	Body weight	Diet	Body weight	Diet
weeks	*grams*	*grams*	*grams*	*grams*
	150		140	
1	160	54	136	31
2	154	46	130	27
3	154	36	124	27
4	153	?	122	?
5	143	?	120	?
6	142	?	115x	?
7	140	?	132	41
8	138	?		
9	128x	?		
10	144	35		
11	152	55		
12	160	46		
13	176	50		

The supply of duhat was exhausted

TABLE 6

Okra; body weight and weekly intake of standard diet

FEEDING PERIOD	ONE GRAM SUPPLEMENT		ONE-HALF GRAM SUPPLEMENT			
	Rat 1 ♀		Rat 13 ♂		Rat 4 ♂	
	Body weight	Diet	Body weight	Diet	Body weight	Diet
weeks	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
	162		177			
	159		159		195	
1	159	44	148	38	192	47
2	138	?	129x	?	178	45
3	118x	?	146	45	178	62
4	142	53	156	48	166	32
5	160	60	167	45	152x	29
6	164	43	174	50	170	47
7	179	47	180	52	180	40
8					178	48
9					186	51
10					204	59

TABLE 7

Artichokes; body weight and weekly intake of standard diet

FEEDING PERIOD	ONE GRAM SUPPLEMENT					
	Rat 23 ♂		Rat 24 ♂		Rat 22 ♂	
	Body weight	Diet	Body weight	Diet	Body weight	Diet
weeks	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
	175		226		205	
	180		224		198	
1	186	62	234	51	184	36
2	191	48	220	39	168x	22
3	182	44	212	28	181	45
4	169	?	195	31	204	62
5	161	36	184x	22	218	64
6	155x	23	197	35	222	
7	177	50	201	36		
8	188	46	204	45		
9	190	54	218	50		
10	190y	48	210y	32		
11	208	59	240	56		

FEEDING PERIOD	ONE-HALF GRAM SUPPLEMENT			
	Rat 15 ♂		Rat 25 ♂	
	Body weight	Diet	Body weight	Diet
weeks	*grams*	*grams*	*grams*	*grams*
	212			
	212		218	
1	196	34	220	70
2	178x	19	224	49
3	181	?	218	38
4	191	44	209	34
5	196	30	195	36
6	196y	?	190	35
7	220	60	178x	31
8			185	50
9			185	48
10			192	40
11			190y	?
12			204	53
13			220	57
14			224	

TABLE 8

Avocado; body weight and weekly intake of standard diet

FEEDING PERIOD	ONE GRAM SUPPLEMENT				ONE-HALF GRAM SUPPLEMENT			
	Rat 7 ♀		Rat 21 ♂		Rat 17 ♂		Rat 20 ♂	
	Body weight	Diet	Body weight	Diet	Body weight	Diet	Body weight	Diet
weeks	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
	141		180		148		148	
	132		187		156		154	
1	121	26	194	49	160	56	153	45
2	112x	?	192	48	158	48	147	42
3	139	44	178	36	147	26	138	26
4	150	56	155x	18	130x	?	120x	20
5	150	42	207	69	160	53	141	42
6	158	54	240	71	178	59	152	49
7			240	61	192	54	167	50
8			244	58	200	48	172	47

FEEDING PERIOD	ONE-TENTH AND FOUR-TENTH GRAM SUPPLEMENTS			
	Rat 16 ♂		Rat 19 ♂	
	Body weight	Diet	Body weight	Diet
weeks	*grams*	*grams*	*grams*	*grams*
	202		156	
			154	
1	172	26	160	51
2	168	31	154	37
3	178	44	154	33
4	176	39	148	27
5	178	41	142	28
6	170x	45	134x	25
7	162	38	130	30
8	154xx	28	128xx	37
9	165	34	142	36
10	160	48	153	43
11	170	47	160	58
12	162y	35	160y	51
13	176	64	184	59
14	200	72		
15	206			

x = 0.1 gram.
xx = 0.4 gram.

was soaked in water for a day, then cooked as is done for culinary use. The mashed mongo including the water in which it was boiled, was then dried, powdered and finally made into pills. Rats declining in weight easily recovered their original weight under a daily supplement of 1 gram to the standard ration free from vitamin B. With ½ gram, one animal recovered its original weight, while two did not in about the same length of time thus indicating that the latter dose is about the minimum for this species.

TOGI: This is the name given to the sprouted mongo. The bean was first soaked in water for 24 hours, then allowed to sprout in the dark for 2 days, cooked, dried including the water used for boiling, and

TABLE 9

Banana flower bud; body weight and weekly intake of standard diet

FEEDING PERIOD	ONE GRAM SUPPLEMENT					
	Rat 27 ♀		Rat 28 ♀		Rat 10 ♀	
	Body weight	Diet	Body weight	Diet	Body weight	Diet
weeks	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
	150		184		126	
1	155	40	186	51	125	32
2	152	38	185	43	122	30
3	154	45	188	51	119	28
4	147	30	178	34	115	27
5	132x	?	161x	26	110	28
6	115y	?	145y	27	110	28
7	145	60	178	67	108	26
8	162	55	196	62	96x	20
					Died	

powdered. Both 1 gram and ½ gram daily supplements caused quick recovery in weight of rats which had been declining on a diet deficient in vitamin B. One-half gram of the togi was more potent than ½ gram of the mongo. This indicates that the vitamin B in the mongo is increased in sprouting; a fact contrary to the finding of Grijns (cited by Chick and Hume (6)) that the anti-beriberi vitamin is lessened in amount as germination takes place. Togi then has three advantages over mongo: greater digestibility (1) and greater content of vitamins B and C (see later).

SWEET POTATO LEAVES: (*Batatas batatas*). The leaves and young shoots of sweet potato are used as vegetables in making stews and simi-

lar dishes. The material for study was obtained dry. One gram daily supplement fed to rats which had been declining in weight on a diet deficient in vitamin B caused immediate recovery. In about the same length of time ½ gram caused only partial recovery. The latter dosage is not as efficient as $\frac{4}{10}$ gram of dried brewery yeast.[4]

TABLE 10

Bamboo shoots; body weight and weekly intake of standard diet

FEEDING PERIOD	ONE GRAM SUPPLEMENT					
	Rat 12 ♂		Rat 15 ♂		Rat 16 ♂	
	Body weight	Diet	Body weight	Diet	Body weight	Diet
weeks	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
	175					
	203		164		202	
1	205	64	178	63	201	74
2	204	65	185	55	202	67
3	188	42	176	37	176	26
4	176	19	158x	?	158x	13
5	157x	10	162	26	168	44
6	172	36	163	41	173	48
7	191	31	172	38	180	41
8	195	46	182	38	172	26
9	196d	43	188xx	42	168xx	31
10	192	42	190	41	178	44
11	170xx	22	196	38	176	39
12	184	44	208	64	178	41
13	198	63	212	54	170y	45
14	198	55				
15	198y	49				
16	210	60				
17	222	68			206	

x = 1 gram.
xx = 2 grams.
d = diet only again.

BILIMBI: (*Averrhoa carambola L.*). Only a small sample in dry condition was obtained, so only the one gram supplement could be tested. After 7 days of 1 gram daily supplement, no improvement in the weight of the animals was found. The intake of basal diet did not

[4] The dried brewery yeast used was kindly furnished by Osborne and Mendel from their stock.

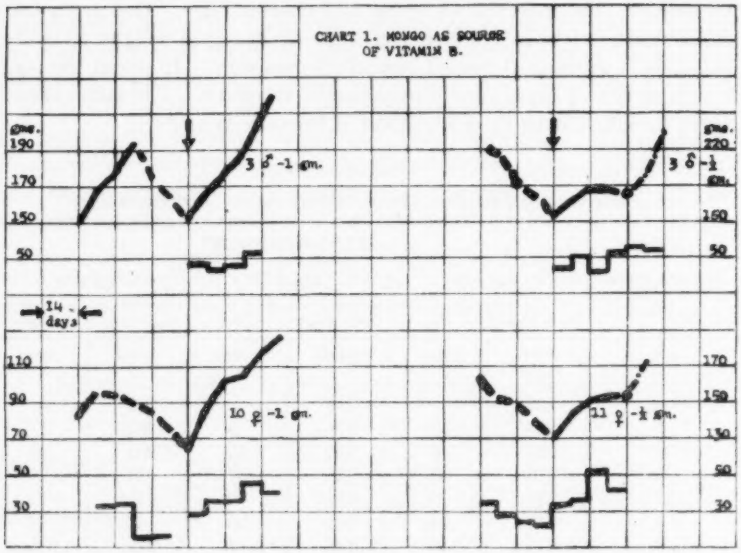


CHART 1. MONGO AS SOURCE OF VITAMIN B.
gms.
190
170
150
50
3 ♂ -1 gm.
3 ♂ -½ gm.
220
160
50
14 days
110
90
70
50
30
10 ♀ -1 gm.
11 ♀ -½ gm.
170
150
130
50
30

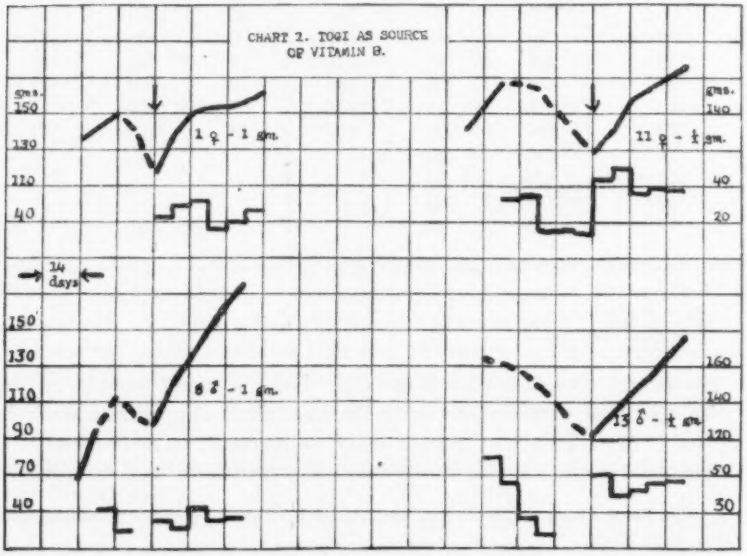


CHART 2. TOGI AS SOURCE OF VITAMIN B.
gms.
150
130
110
40
1 ♀ - 1 gm.
11 ♀ - ½ gm.
140
40
20
14 days
150
130
110
90
70
40
8 ♂ - 1 gm.
13 ♂ - ½ gm.
160
140
120
50
30

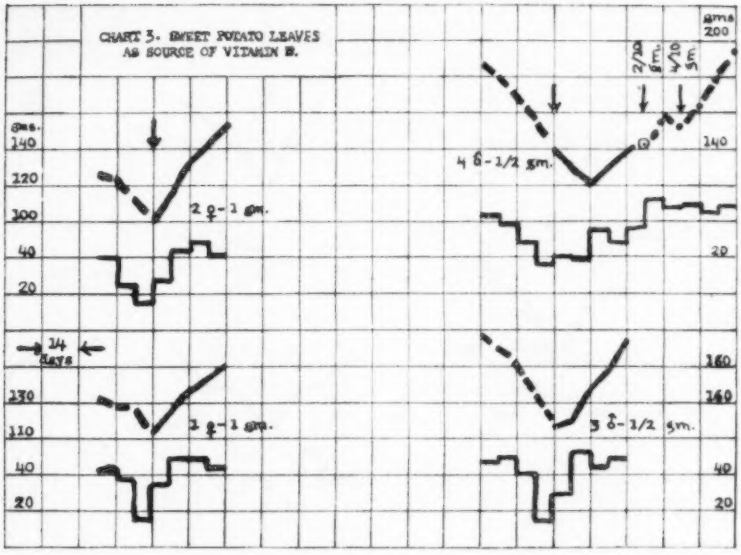


CHART 3. SWEET POTATO LEAVES AS SOURCE OF VITAMIN B.
gms.
140
120
100
40
20
2 ♀-1 gm.
14 days
130
110
40
20
2 ♀-1 gm.
gms
200
2/10 gm.
1/10 gm.
140
4 ♂-1/2 gm.
20
160
150
40
20
3 ♂-1/2 gm.

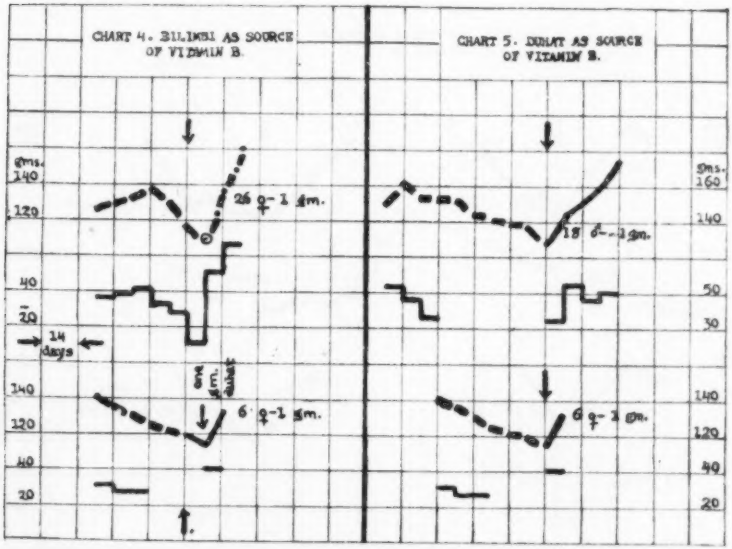


CHART 4. BILIMBI AS SOURCE OF VITAMIN B.
gms.
140
120
40
20
26 ♀-1 gm.
14 days
one gm. dose
140
120
40
20
6 ♀-1 gm.
CHART 5. DURAT AS SOURCE OF VITAMIN B.
gms.
160
140
18 ♂-1 gm.
50
30
140
120
40
20
6 ♀-1 gm.

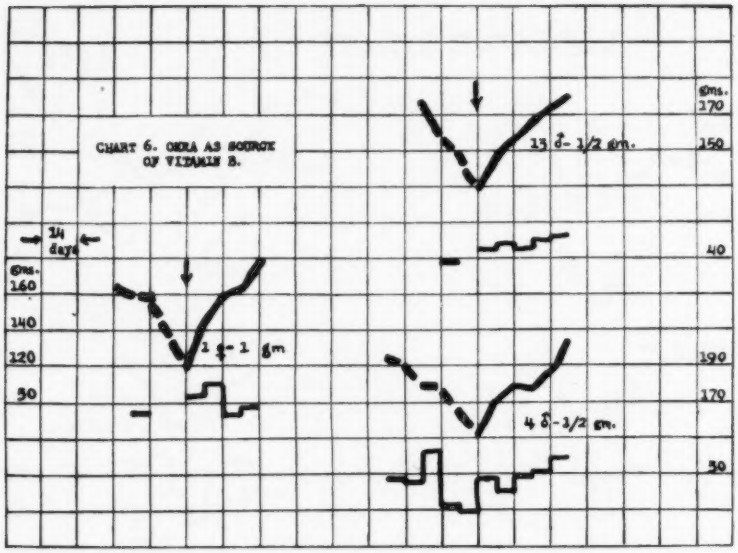


CHART 6. OKRA AS SOURCE OF VITAMIN B.
gms. 170
150
13 δ- 1/2 gm.
14 days
gms. 160
140
120
30
2 δ- 1 gm
40
190
170
4 δ- 1/2 gm.
30

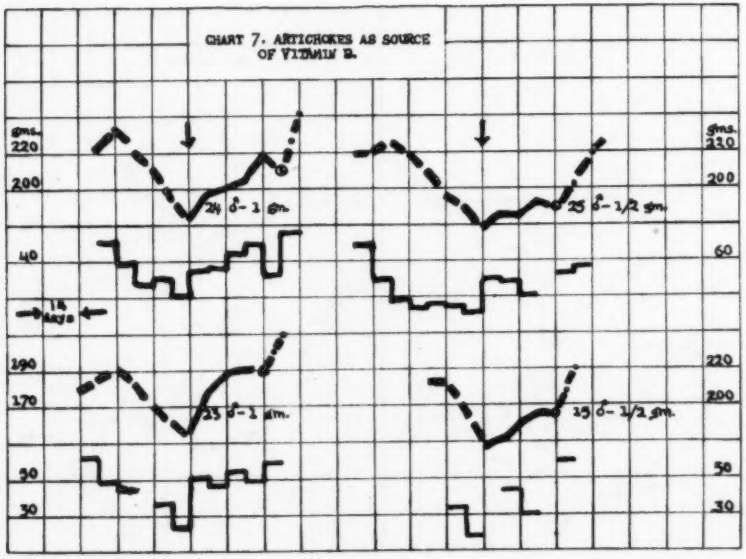


CHART 7. ARTICHOKES AS SOURCE OF VITAMIN B.
gms. 220
200
24 δ- 1 gm.
25 δ- 1/2 gm.
gms. 210
200
40
60
14 days
190
170
23 δ- 1 gm.
23 δ- 1/2 gm.
220
200
30
30
50
30

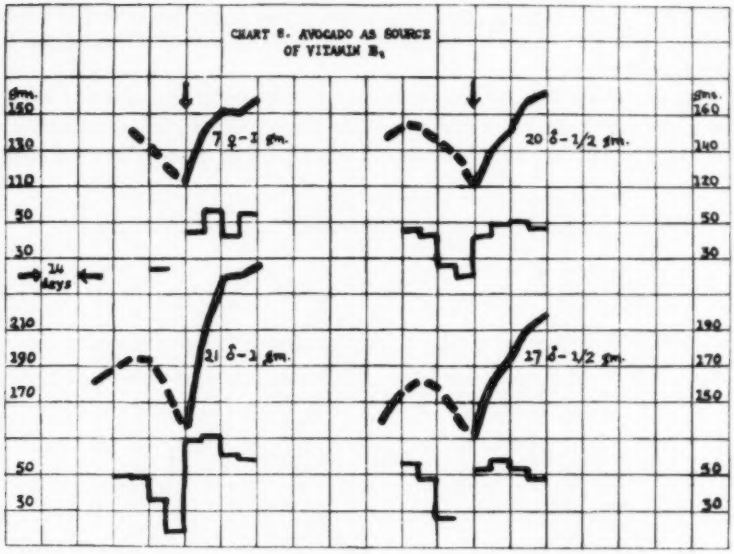


CHART 8. AVOCADO AS SOURCE OF VITAMIN B1
gms.
150
130
110
90
30
7 ♀- 1 gm.
20 ♂- 1/2 gm.
14 days
210
190
170
150
90
30
21 ♂- 1 gm.
17 ♂- 1/2 gm.

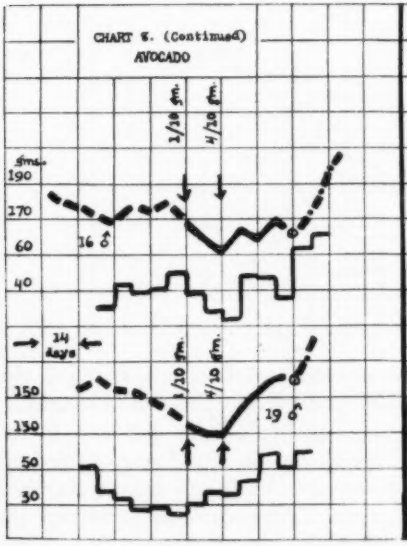


CHART 8. (Continued)
AVOCADO
1/10 gm.
4/10 gm.
gms.
190
170
60
40
16 ♂
14 days
150
130
50
30
19 ♂

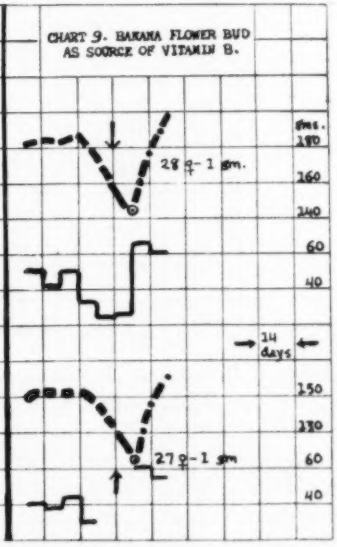
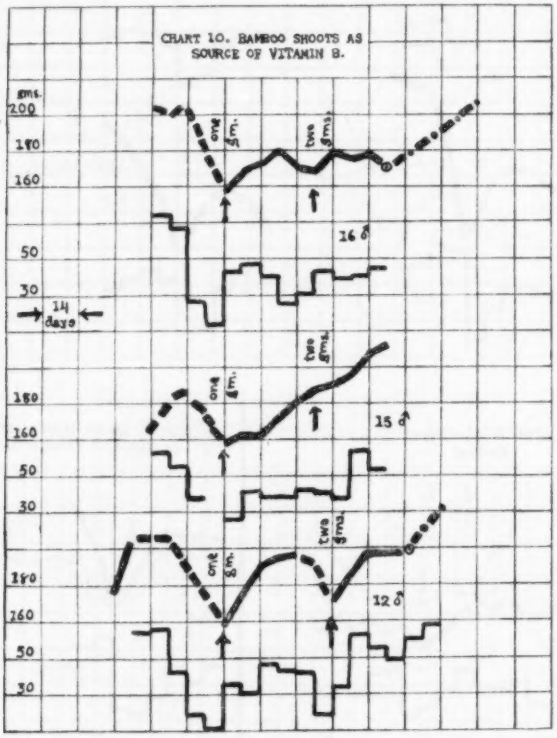


CHART 9. BANANA FLOWER BUD AS SOURCE OF VITAMIN B.
gms.
180
160
140
60
40
28 ♀- 1 gm.
14 days
150
130
60
40
27 ♀- 1 gm

increase. But when $\frac{4}{10}$ gram yeast was given to rat 26, instead of bilimbi, it gained weight very rapidly. Duhat improved the weight and appetite of rat 6.

DUHAT: (*Eugenia jambolana L.*). Only enough material for testing on one animal was obtained. One gram daily supplement caused the recovery of rat 18 which had been declining in weight on a diet



deficient in vitamin B. The incomplete experiment with rat 6 also shows that duhat caused an improvement in appetite and in weight.

OKRA: (*Abelmoschus esculentus*). Although this is not used in the Philippines, it is a tropical plant which can be introduced. The fruit was boiled, dried including the water in which it was boiled, and then powdered. Both 1 gram and $\frac{1}{2}$ gram daily supplements caused the recovery in weight of rats declining due to lack of vitamin B.

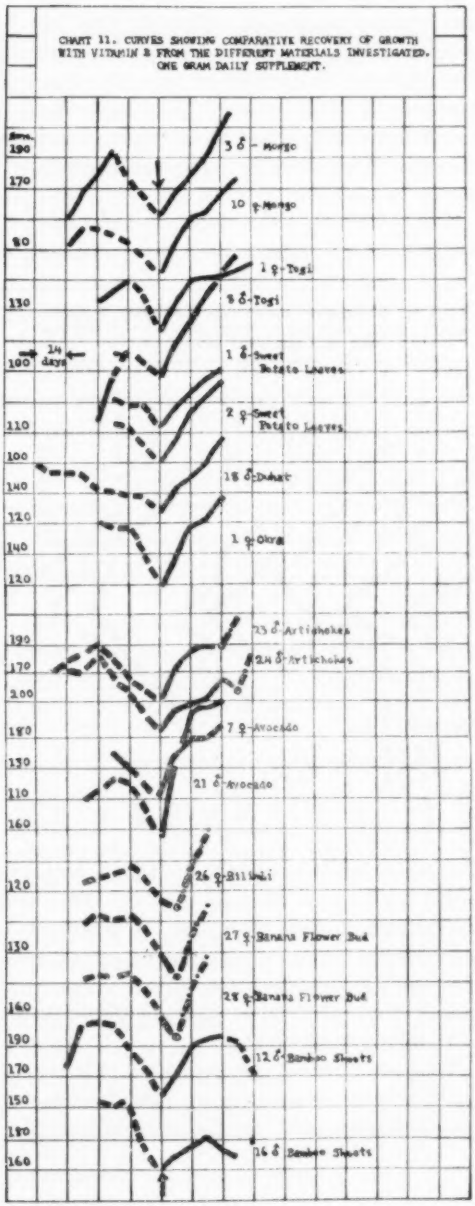
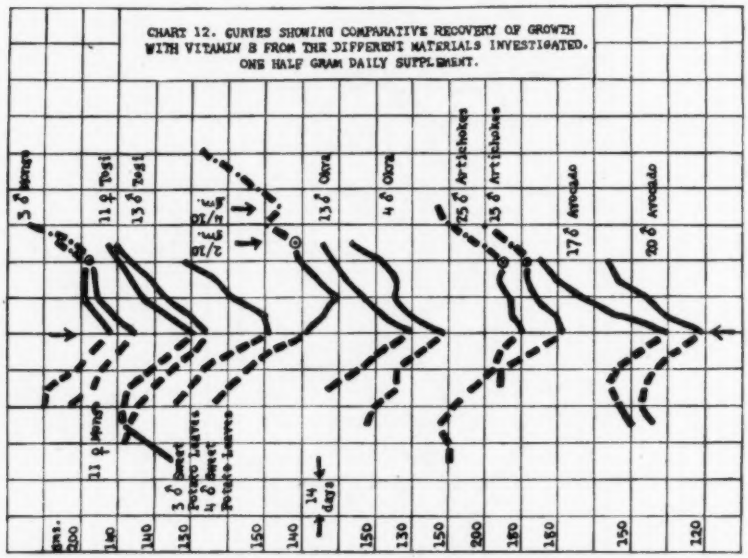


CHART 11. CURVES SHOWING COMPARATIVE RECOVERY OF GROWTH WITH VITAMIN B FROM THE DIFFERENT MATERIALS INVESTIGATED. ONE GRAM DAILY SUPPLEMENT.

ARTICHOKES: (*Cynara scolymus*). Though this has little nutritive value it is eaten as a delicacy. Its use in the Philippines is not known but may be introduced. The edible portion was dried and powdered. The behavior of rats 22, 23, 24 shows that 1 gram daily supplement is probably just enough to cause recovery in weight of rats which had been declining due to lack of vitamin B. One-half gram daily was found to be insufficient.

AVOCADO: (*Persea persea*). This fruit is rich in fat (25), and is being increasingly used not only in the tropics but also in the temper-



ate zones. The edible portion of the dried fruit was ground and extracted with ether to remove most of the fat. Rats which had been declining in weight due to lack of vitamin B, easily recovered on the administration of either 1 gram or $\frac{1}{2}$ gram extracted avocado daily in addition to the standard ration. One-tenth gram was found to be insufficient; and $\frac{4}{10}$ gram seems to be just enough to cause maintenance but not complete recovery of weight.

BANANA FLOWER BUD: (*Musa sapientum*). This is used in making salads. The sample was obtained in an already dried condition and the variety was undetermined. In the Philippines only the buds of

certain varieties are used, because some have bitter taste. Even a 1-gram daily supplement was not sufficient to check the decline in weight of rats which had been taking vitamin B free diet. Similar results were obtained with mice, which could not even be maintained by daily addition of $\frac{1}{2}$ gram of banana flower bud to the basal diet.

BAMBOO SHOOTS: (*Bambusa sp.*). Canned material from Japan was used. The shoots including the water in which they were boiled were dried and powdered. Rat 15 after 5 weeks of 1 gram daily supplement to the basal diet recovered its weight, and when in addition to this supplement alcoholic extract of the powdered shoots was incorporated with the basal diet the animal continued gaining in weight. Enough of the extract was added to the basal diet so that an amount corresponding to approximately 1 gram of the original shoot was eaten by the rat daily in addition to the 1 gram daily supplement given separately. The alcoholic extract was first offered separately also but was refused by the animal. Rat 16 after 5 weeks of 1 gram daily supplement did not recover its original weight. Then a 2-gram daily supplement was given. Neither weight nor appetite improved. Oftentimes the rat was observed to have eaten only the bamboo supplement and very little of the standard food. This is a case in which the limited capacity of the animal prevents it from eating more of the necessary basal diet when the bulky food containing the vitamin was preferred. It finally recovered its weight on the administration of $\frac{4}{10}$ gram yeast daily. Rat 12 did not fully recover its weight after about the same length of time. To determine whether there is really vitamin B in the bamboo shoots, the rat was placed on the basal diet again. Weight and appetite both declined. Then 2-grams daily supplement of bamboo was added to the basal ration. A result similar to that with rat 16 was obtained.

One gram daily was also given to mice which had been declining in weight due to lack of vitamin B in the diet, but no improvement was noticed.

On the assumption that the vitamin B and the antineuritic vitamin are related, an experiment was tried with pigeons. Three pigeons (nos. 1, 2 and 3) were stuffed with polished rice, equal in weight to from $\frac{1}{20}$ to $\frac{1}{12}$ of their body weight, plus 6 grams of powdered bamboo shoots daily. All died of atrophic polyneuritis (13), pigeons 1 and 2 after 18 days, and pigeon 3 after 35 days. Of the three controls without bamboo, pigeon 4 died of polyneuritis after 12 days; pigeon 5 on the 19th day showed a tendency to fall on one side (a sign of polyneu-

ritis) and was cured by the injection of an alcoholic extract from 6 grams of wheat embryo. Control pigeon 6 died of polyneuritis after 31 days. Pigeons 3 and 6 always vomited the food that was stuffed in them except during the 4 or 5 days before their death. It thus appears that bamboo shoots have little vitamin B.

TAMARIND: (*Tamarindus indicus*). The young leaves are used as vegetables and give sour taste to the broth. The rats could not be made to eat the material. Even incorporating the powdered leaves with the basal diet was unsuccessful. Neutralization of the sour taste with sugar was tried but too much sugar was required and thus the material became bulky. The alcoholic extract was also refused by the rats.

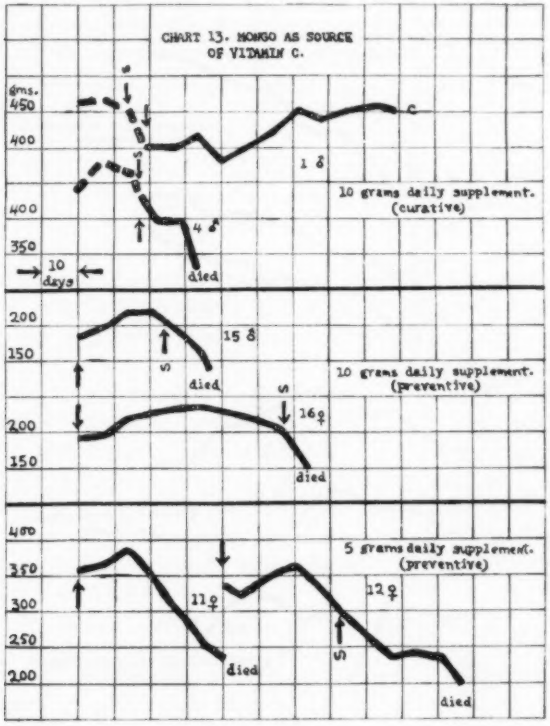
PIGWEED: (*Cycloloma platyphyllum*). This is also used in making stews and gives bitter taste to the broth. Like the tamarind, it was distasteful to the rats.

II. TEST FOR VITAMIN C

Scurvy, like beriberi, is found in the Philippines, being especially common among children under 2 years of age. Poor nutrition is the cause of this. Milk is only within the reach of a few. Cases are common in which mothers who have poor or little milk give the broth of boiled polished rice to the children as a substitute for milk. This milk substitute is of course poor in both vitamins B and C. Since vitamin C is destroyed or diminished in potency under the influence of heat, as in cooking and drying (22), only those materials which promised useful results were tested. The protocols of body weights are given in graphic form in the appendix (charts 13 and 14).

Method: Guinea pigs were used as the experimental animals. Hess and Unger's (23) scorbutic diet of hay, oats, plus 0.5 to 1 cc. of cod liver oil daily and water ad libitum was used as the basal diet in most of the tests. The oil was given by a pipette. At first finely chopped hard boiled egg (yolk plus white) was offered instead of the cod liver oil, but the animals would not eat the former. In the experiment with avocado no use of cod liver oil was made. On the basal ration control guinea pigs (nos. 2, 13 and 14) succumbed to scurvy after 16, 26 and 27 days respectively. In all of the guinea pigs autopsied it was found that the hemorrhages were more severe in those that were not given oil than in those animals that ate the improved diet. At first curative experiments were tried; but it was difficult to make the guinea pigs eat when they were already suffering from scurvy and therefore preventive

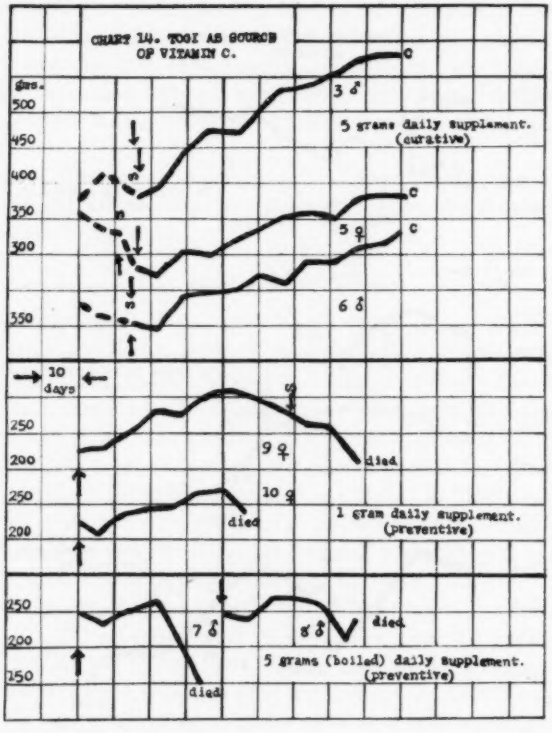
tests were performed. In the curative experiments the animals demonstrated the externals signs of scurvy such as the scorbutic position (22), crying when handled, painful or swollen limbs, and pale gums, before the materials to be tested were given as supplement to the scorbutic diet.



DISCUSSIONS OF THE MATERIALS USED AND THE FEEDING EXPERIMENTS. Inasmuch as in every day nutrition the materials to be tested here are never eaten alone, since the amount that an individual can eat in a day is dependent in part on his alimentary capacity, and since, further, our object was to get a comparative estimate of the possible value of the foods as practical sources of vitamin C, a daily supplement of 10 grams to each guinea pig in addition to the basal ration was considered the maximum.

MONGO: (*Phaseolus mongo L.*). In all cases the bean was ground and soaked in water, to make it more palatable, before being offered to the animals.

Ten grams daily curative supplement. Guinea pig 4 began to drag the hind legs after 17 days of scorbutic ration. Mongo was now given



separately as supplement. The decline in weight continued and on the 33rd day it died of scurvy.

Guinea pig 1 showed external symptoms of scurvy on the 14th day of scorbutic diet. When mongo was given, the animal improved in appearance and recovered the original weight. On the 89th day it was chloroformed and autopsy showed little hemorrhage in the right hind leg.

Ten grams daily preventive supplement. Guinea pig 15 never ate all of the mongo. After 25 days, the animal had difficulty in moving the hind legs, and on the 36th day it died of scurvy.

Guinea pig 16 did not eat all of the mongo for the first 2 weeks. On the 57th day the animal began dragging the hind legs, and on the 64th day it died. Autopsy showed the signs of scurvy.

Five grams daily preventive supplement. Guinea pig 11 died on the 40th day without showing external sign of scurvy except pale gums. Autopsy showed slight hemorrhages at the leg joints.

Guinea pig 12 on the 33rd day cried when the left hind leg was touched. Soon an eye became blind and on the 67th day it died of scurvy.

Togi: Fürst (14), Chick and Hume (6), Cohen and Mendel (7), Chick and Delf (5) and others have shown that the vitamin C of dried peas and lentils is much increased in germination. Wiltshire (36) has successfully used germinated haricot beans which were boiled for 10 minutes in the treatment of human scurvy. Gerstenberger (15) found that malt soup extract prepared from malt sprouted in a certain way has high antiscorbutic value. Recently it has been shown that oxidation (10) is the chief factor in the destruction of this vitamin. Evidently the method of cooking has something to do with the destruction of vitamin C. Adkins (1) has found that germinated bean is easier to digest than the ungerminated. In the light of the above findings togi ought to be of great value in nutrition. It is cheap and at the same time easily obtainable. In anticipation of its high content of vitamin C, 5 grams daily supplement to the scorbutic diet was first tried. The togi used was weighed and given to the animals without the outer seed coat.

Five grams daily curative supplement. Guinea pig 3 having developed external signs of scurvy on the 16th day of scorbutic diet was given fresh togi daily as supplement. It gradually improved in appearance and increased in weight over that of the original. On the 89th day it was chloroformed and no sign of scurvy was shown by autopsy.

Guinea pig 5 showed signs of scurvy on the 13th day of scorbutic diet, whereupon it was given fresh togi daily as supplement. It gradually recovered the original weight. On the 91st day it was chloroformed. Autopsy showed nothing abnormal.

Guinea pig 6 on scorbutic diet developed signs of scurvy on the 15th day and was then given fresh togi as supplement. On the 90th day it weighed 100 grams over the initial weight. When autopsied after being chloroformed no sign of scurvy was found.

One gram daily preventive supplement. Guinea pig 9 was given togi as supplement in addition to the scorbutic diet. On the 60th day it began dragging the hind legs, and on the 78th day died of severe scurvy.

Guinea pig 10 was treated the same as guinea pig 9. On the 46th day it died without external sign of scurvy. Slight hemorrhages at the limbs were seen when autopsied.

Five grams boiled (10 minutes) togi daily preventive supplement. Guinea pig 7 died of scurvy after 34 days on the scorbutic diet, plus boiled togi daily. Guinea pig 8 on the same food as guinea pig 7 died of scurvy on the 38th day.

To both animals the water in which the togi was boiled was also offered. Care was taken to boil the togi in the least amount of water possible, and boiling was conducted in a covered vessel as is done in every day cooking.

AVOCADO: (*Persea persea*). This fruit is eaten raw, and if it could be shown to be high in vitamin C, it would be of great value. Unfortunately the guinea pigs would not eat the avocado; so both the preventive and curative experiments were unsuccessful.

SUMMARY

Several plant foods were tested for their content of vitamins B and C.

Togi, okra and *avocado* were found to be comparatively high in vitamin B. One-half gram of each of them as daily supplement to the standard vitamin B free diet caused the recovery in weight of rats which had been declining because of lack of this accessory food factor.

Mongo, sweet potato leaves and duhat contain enough vitamin so that 1 gram of them as daily supplement caused the recovery in weight of rats which had been declining due to lack of vitamin B.

Artichokes, bilimbi, banana flower bud and bamboo shoots are relatively poor in vitamin B.

The vitamin B in mongo was increased in germination, a fact contrary to the finding of Grijns (19) that the anti-beriberi vitamin is lessened in amount as germination takes place. Mongo is relatively poor in vitamin C.

Togi when fresh is relatively rich in vitamin C; but after it is prepared for culinary use, the vitamin C is destroyed.

The observation of several investigators that vitamin C is increased when peas, lentils and beans are germinated, has been verified in the

case of mongo. Ten grams of mongo as daily supplement to the scorbutic diet failed to protect guinea pigs from scurvy, while 5 grams of fresh togi as supplement to the same scorbutic diet cured 3 guinea pigs of the disease.

I desire to express my hearty thanks to Prof. Lafayette B. Mendel for suggesting this subject to me, for his help in securing the materials investigated, and for advice and criticism during the progress of the work. Part of the expenses was defrayed by a grant from the Russell H. Chittenden Research Fund for Physiological Chemistry.

BIBLIOGRAPHY

(1) Adkins: Biochem. Journ., 1920, xiv, 637.
(2) Agcaoili: Philip. Journ. Sci., 1916, xi (A), 91.
(3) Brill and Alincastre: Philip. Journ. Sci., 1917, xii (A), 127.
(4) Cajori: Jour. Biol. Chem., 1920, xliii, 583.
(5) Chick and Delf: Biochem. Journ., 1919, xiii, 199.
(6) Chick and Hume: Tr. Soc. Trop. M. and Hyg., 1917, x, 141.
(7) Cohen and Mendel: Jour. Biol. Chem., 1918, xxxv, 425.
(8) Coward and Drummond: Biochem. Journ., 1920, xiv, 665.
(9) Denton and Kohman: Journ. Biol. Chem., 1918, xxxvi, 249.
(10) Dutcher, Harshaw and Hall: Journ. Biol. Chem., 1921, xlvii, 483.
(11) Eddy: Bibliographic review, 1756. *Abst. Bact.*, 1919, iii, 313.
(12) Ferry: Journ. Lab. Clin. Med., 1920, v, 735.
(13) Funk: Die Vitamine, 1914, pub. by J. F. Bergmann, 23.
(14) Furst: Zeitschr. Hyg., 1912, lxxii, 121.
(15) Gerstenberger: Amer. Journ. Dis. Child., 1921, xxi, 315.
(16) Givens and Cohen: Journ. Biol. Chem., 1918, xxxvi, 127.
(17) Givens and McClugage: Journ. Biol. Chem., 1919, xxxvii, 253.
(18) Givens and McClugage: Journ. Biol. Chem., 1920, xlii, 491.
(19) Grijns: cited by Chick and Hume (6).
(20) Harden and Robinson: Biochem. Journ., 1920, xiv, 171.
(21) Harden and Zilva: Biochem. Journ., 1918, xii, 259.
(22) Hess: Scurvy, past and present, 1920.
(23) Hess and Unger: Journ. Biol. Chem., 1918, xxxv 479.
(24) Hopkins and Chick: Bull. no. 38, Medical Research Committee, England, 1919.
(25) Jaffa and Albro: Ann. Rept. Cal. Avocado Assoc., 1917, 85.
(26) Johns, Finks and Paul: Journ. Biol. Chem., 1919, xxxvii, 497.
(27) McCollum: The Harvey Lectures, 1916-07, xii, 151.
(28) McCollum: Journ. Amer. Med. Assoc., 1917, lxviii, 1379.
(29) McCollum, Simmonds and Parsons: Journ. Biol. Chem., 1918, xxxvi, 197.
(30) Osborne and Mendel: Journ. Biol. Chem., 1919, 37, 187.
(31) Osborne and Mendel: Journ. Biol. Chem., 1919, xxxix, 29.
(32) Osborne and Mendel: Journ. Biol. Chem., 1920, xxxxi, 451.

(33) OSBORNE AND MENDEL: Journ. Biol. Chem., 1920, xxxxii, 465.
(34) SUGIURA AND BENEDICT: Journ. Biol. Chem., 1918, xxxvi, 171.
(35) WHIPPLE: Journ. Biol. Chem., 1920, xxxxiv, 175.
(36) WILTSHIRE: Lancet, 1918, (2), 811.

Key to tables

x = feeding of source of vitamin B as daily supplement was begun.
y = $\frac{4}{10}$ gram yeast replaced other vitamin supplement.
? = intake of standard diet could not be measured because the animal scattered the food.
The weekly intake of standard diet does not include the vitamin supplement.

Key to charts

S = external sign of scurvy was first detected.
C = animal was chloroformed.
· = $\frac{4}{10}$ gram yeast replaced other vitamin supplement.
---------- = growth on standard diet alone.
———— = growth on standard diet plus vitamin supplement other than yeast.
—·—·—·— = growth on standard diet plus yeast.
The arrow, unless labeled otherwise, indicates the point where the feeding of source of vitamin B or C was begun.
The weekly intake of standard diet is represented in graphic form under each curve of weekly body weight.

THE EFFECT OF VITAMINE DEFICIENCY ON VARIOUS SPECIES OF ANIMALS

II. Observations on the Comparative Vitamine A Requirement of Rabbits, Rats, Swine and Chickens[1]

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The first paper of this series (1) reported the production of xerophthalmia in the rabbit and suggested that the requirement of the rabbit for fat-soluble vitamine (Vitamine A) is greater than that of the rat. Data will be presented in this paper which demonstrate this assumption to be a fact and point toward the possibility that this difference in vitamine requirement may include the herbivora in general, which normally subsist on materials such as green leaves, which are richer in vitamine than the dietary of the omnivora.

Considerable interest has been manifested in the literature of late regarding the comparative vitamine requirements of various species, although until recently the rat was the only mammal used to any considerable extent in work on the vitamines. Some quantitative work on the minimum Vitamine A requirement of the rat has been carried out, but the application of these data to other species and to the human family can be made only with great caution. The results which we are obtaining in the present study on several species of animals suggest that with a further knowledge of the vitamine requirements of animals of different types of feeding habits it will be possible to forecast more accurately the requirements of other species of economic importance as well as of the human family, which are not easily used in experiments of this nature.

Experimental. A ration similar to that which was employed in our previous work with rabbits has been used to compare rabbits and rats. Its composition was as follows:

[1] Published with the permission of the Director, Iowa Agricultural Experiment Station

		Salt mixture no. 1	
Commercial casein	20	NaCl	0.45
Dextrin	70	$MgSO_4.7H_2O$	1.10
Salts	5	$NaH_2PO_4.H_2O$	0.85
Wheat embryo	5	K_2HPO_4	2.40
Alcohol-extracted alfalfa	25	$CaH_4(PO_4)_2.H_2O$	1.35
	125	Ca lactate	3.00
		Fe citrate	0.25
		KI	trace

The alfalfa meal used had been extracted with hot alcohol for 96 hours to remove the fat-soluble vitamine. This ration was assumed to be complete except for Vitamine A, a small amount of which was, however, present in the casein and the wheat embryo. Inasmuch as this ration was to be used for a comparative study of the relative requirement of these two species, we were not concerned at the outset with the removal of the last traces of Vitamine A. Indeed, the typical growth curves presented in chart 1 show definitely that the ration contained enough of this vitamine to support slow growth and maintenance over a considerable period of time in the rat, without successful reproduction. These rats showed a remarkable growth without the appearance of xerophthalmia on a ration which was very low in Vitamine A. In this connection Osborne and Mendel (2) have recently reported, while our experiments were still in progress, that their rats made normal growth when only 0.1 gram of butter-fat was added per day to a carefully purified ration. Judging from the growth curves of the animals our ration, which included commercial casein, contained some Vitamine A, but not nearly so much as would be contained in the successful rations used by Osborne and Mendel. This small amount of Vitamine A in our ration, however, which produced slow growth in the rat, was not sufficient to keep a rabbit alive, as shown in chart 2. Xerophthalmia developed in every case after about 4 weeks on the ration, just as in the cases reported in the first paper of this series. Rabbits varying in age from 3 to 8 weeks were used on the same ration, and the time required for the development of xerothphalmia varied from 2 to 8 weeks, in direct proportion to the age of the animal when put on the ration. We are continuing the study of the varying susceptibility with age to nutritive deficiencies.

Another ration consisting of oats 60, gelatin 10, agar 5, salts 5, dextrin 20, extracted alfalfa 20, did not produce normal growth in rabbits 6 and 7 although the ration was complete in all respects except its low Vitamine A content (3). This ration produced a chronic form of xeroph-

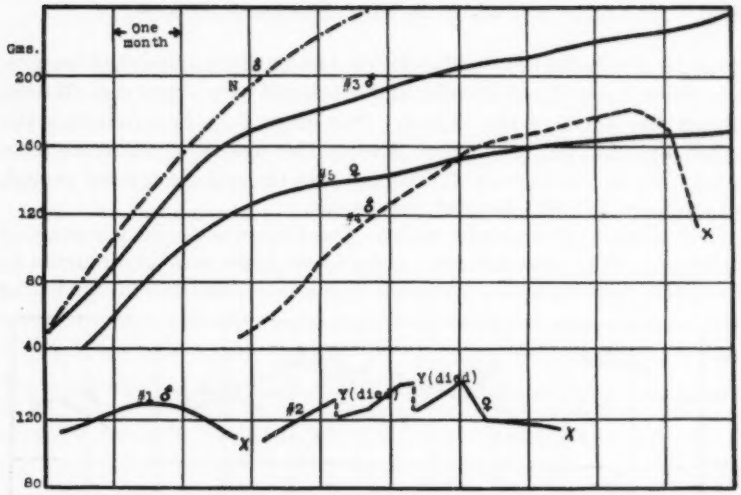

Chart 1. Growth of rats on commercial casein ration which was also fed to rabbits and chickens. Rat 3 died one month later than end of curve. Rat 5 died two months later. Normal growth curve indicated by *N; Y* indicates birth of young.

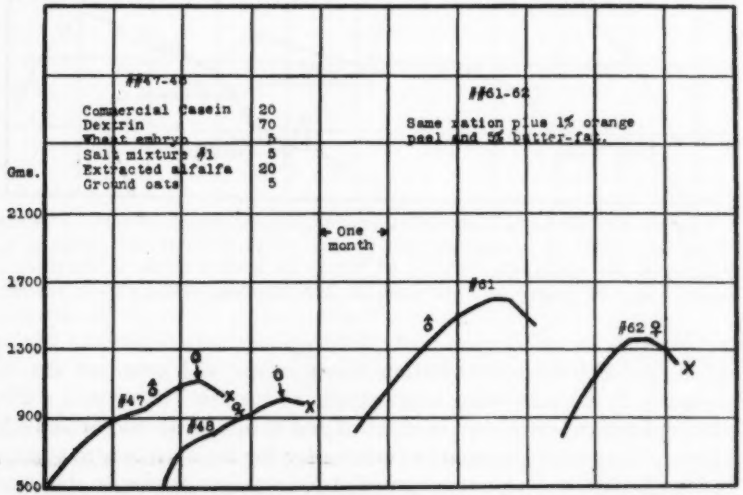

Chart 2. Rabbits 47 and 48. Typical curves obtained on commercial casein ration. Compare growth curves of rats on same ration in chart 1. *O* indicates xerophthalmia. Rabbits 61 and 62. Failure to grow on "complete" semi-purified ration.

thalmia, which did not develop in one case until the rabbit had been on the ration more than 3 months, and continued over a period of 60 days before the death of the animal. (See chart 3.) This indicates the possibility that the rabbit may develop this disease in a chronic form when there is a deficiency of Vitamine A in the ration not great enough to produce the acute form of the disease.

The question of scurvy in rabbits. Our first ration, which contained extracted alfalfa, was deficient, as far as we knew, only in Vitamine A, from observations on the nutrition of the rat. This ration was fed to

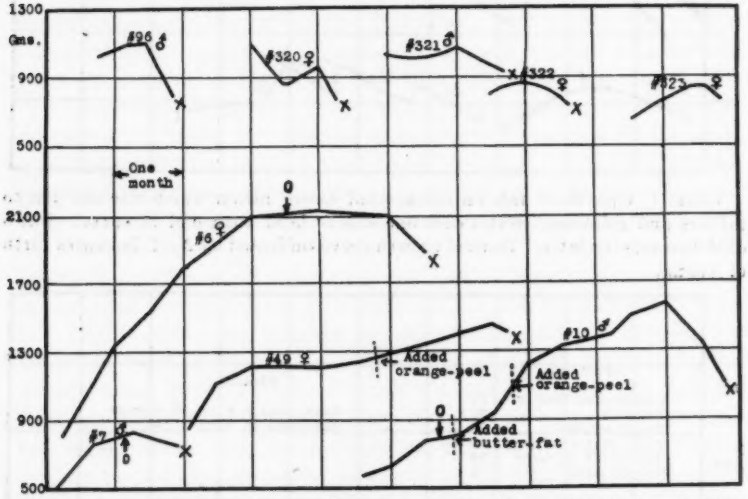

Chart 3. Rabbits 6 and 7, oats-gelatin ration, chronic xerophthalmia. Rabbits 10 and 49, failure on commercial casein ration plus butter-fat and orange peel. *O* indicates xerophthalmia. Rabbit 96, failure on purified casein 18, dextrin 62, salts 5, paper 10, yeast 5. Rabbits 320, 321, 322, 323, same ration plus butter-fat.

rabbits with the addition of butter-fat, equivalent to 5 per cent of the ration, and also with the addition in some cases of 1 gram per day of orange peel, dried at room temperature, to furnish Vitamine C. The curves of growth are shown in charts 2 and 3, rabbits 10, 49, 61 and 62. These rabbits were intended as controls for the ration which was used, as described above, to produce xerophthalmia and to determine the comparative Vitamine A requirement of the rabbit. In no case, however, have we yet been able to bring a rabbit to maturity on this semi-purified ration, plus butter-fat and orange peel.

A similar failure to grow on a purified casein ration so supplemented

as to be satisfactory for the rat is illustrated in chart 3, rabbits 320, 321, 322 and 323. The ration used is shown on the chart.

These rations, without the orange peel, are perfect as far as the nutrition of the rat is concerned. Since the rabbit, however, requires considerably more Vitamine A than the rat, the fact that it does not grow on the above rations leads us to believe that there may be still other ways in which its nutritional requirements are different than those of the rat. We are now studying this matter thoroughly with a view to determining the defects of this ration for the nutrition of the rabbit. Whether or not another vitamine is necessary in this case, as suggested by Hart, Steenbock and Hoppert (4), it seems evident from our observations thus far, that something is lacking in this ration for the satisfactory nutrition of the rabbit. In this connection we consider that the nutritional requirements of the rabbit are important in that they typify the requirements of herbivorous animals better than does the rat, which belongs to the omnivorous type. As mentioned above, we are considering the possibility of the effect of the feeding habits of omnivora, herbivora and carnivora upon their vitamine requirements. This is leading our work away from the qualitative and toward the quantitative aspect of the fundamental requirements of various species. The study of the nutrition of the rabbit enables us to approach the goal of a more accurate knowledge of the requirements of other herbivora which are not convenient laboratory animals. We have also found points of similarity in the nutritive requirements of rats and swine, both of the omnivorous type.

In attempting so to modify our purified ration as to permit growth in the rabbit, the following interesting observations were made. Rabbit 10 developed xerophthalmia and was cured by addition of butter-fat to the ration. A little later 1 gram daily of air-dried orange peel, which we have used successfully to prevent scurvy in the guinea pig, was also added to the ration, with slight improvement in appetite and growth. This was done because of the tendency in the literature to postulate scurvy as the cause of some of the evidences of malnutrition which have been observed in the rabbit, particularly on an exclusive oats ration. However, the orange peel did not prevent ultimate nutritive failure and death after 4 months on the presumably complete ration, as shown in chart 3. A peculiar weakness or malformation of the hind legs which we have observed in nearly all our animals on these purified rations, was observed in this animal (no. 10) for $2\frac{1}{2}$ months before its death. This condition was somewhat suggestive of rickets.

Rabbit 49 (chart 3) was kept on the same ration plus butter-fat for nearly 5 months, growing very slowly. During the last 2 months orange peel was also added to the ration, but the same condition of the hind legs developed. Moreover, during the last 3 or 4 days of life this animal remained in an entirely characteristic scurvy posture and died in that position. During this time the animal ground its teeth as scorbutic guinea pigs do. The gums, however, were not congested. At autopsy the bones were found very brittle, but the ribs were not beaded. Rabbits 61 and 62 also failed on this presumably complete ration.

With the intention of determining definitely whether or not a rabbit is susceptible to scurvy, rabbits 63, 64, 78, 79 and 109 were placed on rations which are known to produce scurvy in the guinea pig very quickly, but which were otherwise satisfactory. The first two were given equal parts of oats and alfalfa meal which had been heated to 70° in an electric oven for 4 to 5 days. This ration was moistened and fed daily. Although unappetizing, the ration was efficient enough to produce growth at approximately the normal rate from 700 to about 2800 grams in 4 months. When taken off the experiment at the end of 8 months both animals were in excellent condition.

Rabbits 78 and 79 were given a soy-bean ration used by Parsons (5) which was composed as follows:

Autoclaved soy-beans	84
NaCl	3
Calcium lactate	3
Yeast	3
Butter-fat	3
Paper	2

This ration was so supplemented that it was complete except for Vitamine C, and was reported by Parsons to cause scurvy in guinea pigs very quickly. Our rabbits ate enough of the shavings litter to furnish sufficient roughage, and grew at a rapid rate, as shown in chart 4. When taken off the experiment they were in fine condition and still growing. In the case of rabbit 109 (chart 4) slow growth over a considerable period was obtained in a ration of alfalfa meal autoclaved 3 hours at 15 pounds pressure, and distilled water. The ultimate failure of this animal was associated with no signs of scurvy. Furthermore, our rabbits on a ration of oats, salts and butter-fat have in some cases lived a period of several months and have never shown signs of scurvy. Rabbit 111 showed remarkable growth on this ration and is still alive after having been on this ration 14 months. Inasmuch as we were

unable to produce scurvy in rabbits on these rations, and since the nutritive failure of rabbits sometimes designated in the literature as scurvy has been produced on incomplete rations such as oats, we are of the opinion that rabbits are not susceptible to this disease, but that the disturbances noted have been of a different nature.

Paralysis in rabbits. When feeding rabbits oats alone and similar rations, we have observed nutritive failure, generally terminating in a peculiar paralysis of the hind quarters. This condition is somewhat

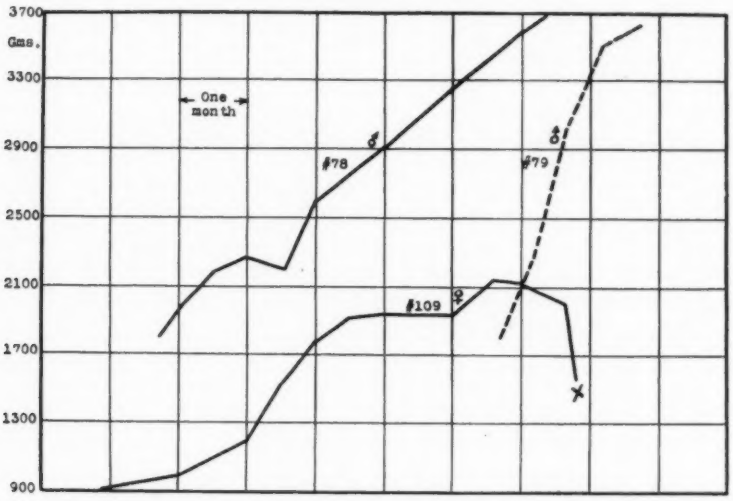

Chart 4. Rabbits 78 and 79, autoclaved soy-bean ration. Rabbit 109, alfalfa autoclaved three hours at 15 pounds pressure. This rabbit showed no signs of scurvy.

similar to the weakness of the hind legs which developed in our rabbits 10 and 49 and others mentioned above, on purified rations, but we are convinced it has nothing to do with scurvy. Rabbits 39, 40, 117 and 118 were fed rations of ground oats, supplemented only with sodium chloride and calcium carbonate. Nos. 39 and 40 both showed symptoms of paralysis after $2\frac{1}{2}$ months, but no. 39 recovered and no. 40 died. Rabbits 117 and 118, on the same ration, became paralyzed in the hind quarters after about $2\frac{1}{2}$ months on the ration, and failed to recover, although each was fed lettuce and cabbage after paralysis occurred. A photograph of rabbit 118 is shown in figure 1. Rabbit

114 also became paralyzed in just the same manner after nearly 4 months on a ration of corn grain plus sodium chloride, calcium carbonate and 5 per cent of butter-fat. The butter-fat did not prevent the paralysis in this case, although in all the other cases we tried it did so whenever added to the oats ration. This paralysis is perhaps not an evidence of a specific deficiency, although we cannot be certain upon that point as we have not yet tried supplementing the above rations with yeast. This unique manifestation of the deficiencies of oats and corn for rabbits is being investigated further.

In connection with the question of various symptoms of deficiencies of certain rations, we have observed that it is a very difficult problem at times to ascribe certain specific symptoms of a faulty diet to a partic-

Fig. 1. Rabbit 118, showing paralysis of the hind quarters developed on a ration of ground oats plus 1 per cent NaCl and 1 per cent $CaCO_3$.

ular factor which might be lacking in that diet. We have noticed in many of the rats in our colony various diseased conditions of the skin, in some cases so marked as to cause growths and wart-like malformations on the ears and nose and characteristic concretions upon the tail. In a good many cases these eruptions have been cured by the feeding of butter-fat, in other cases by the addition of casein to the ration, so that it appears that these diseased conditions of the skin may be the outcome of a poor nutritive condition and not due to any specific factor.

The Vitamine A requirement of growing pigs. Inasmuch as reports have come to the authors of the occurrence of sore eyes and blindness in suckling pigs it became of interest in connection with the work reported here to determine the susceptibility of pigs to xerophthalmia, especially

during the suckling period. We have fed both suckling pigs and suckling rabbits on skimmed milk containing 0.1 per cent fat or less for periods of 7 weeks and 8 weeks respectively with no signs of xerophthalmia.

In order to determine whether a deficiency of Vitamine A in the ration of the sow would cause eye-disease in the young pigs we fed a sow throughout the gestation and suckling periods on the following ration:

White corn (6)	55
Linseed oil meal	22
Ground oats	15
Tankage	5
Supplementary salt mixture	3

No green vegetation was obtainable by the sow or pigs. Four pigs were farrowed, three of which lived but remained rather unthrifty and grew slowly both during the suckling period and after, when they were fed the same ration as the sow had received. No signs of congestion of the conjunctivae were observed at any time. After several months on this ration an allowance of about 10 grams of butter-fat per day was given to the smaller and less thrifty animal, which was nearly dead. This pig improved rapidly in condition while the other continued to show the decided effect of the vitamine deficiency, but no sign of xerophthalmia. From our experience with this ration, not all detailed here, we are inclined to class the pig with the rat in that it probably requires less Vitamine A than the rabbit. Its need for this particular vitamine, however, is here demonstrated to be great enough to make the question of practical importance where pigs are fed without access to pasture or forage. The white corn ration noted above was made up from common feeding materials and so constituted as to supply, with its supplementary salt mixture, good quality of protein and all other dietary essentials except that it was deficient in Vitamine A.

The same ration was fed to both rabbits and rats, to make possible further comparisons between these species. Its marked deficiency in Vitamine A was demonstrated by the fact that it quickly caused xerophthalmia and death in rabbit 81 after 6 weeks on the ration. Rabbit 80 showed slight xerophthalmia at the same time, but was cured and grew normally thereafter with the addition of 5 per cent butter fat to the ration. Four rats on the same ration grew fairly well showing growth curves slightly below the normal, and reached maturity, but the young which were born were not suckled and died in a few hours or were eaten, a characteristic effect of deficient rations. This com-

parison shows again the similarity of the Vitamine A requirements of pigs and rats, and the larger requirements of rabbits.

Preliminary experiment with chickens. As far as known to the authors, xerophthalmia has never been positively produced in chickens. Wilkins and Dutcher (7) have recently reported one cockerel which developed on a rice diet inflamed eyes which were apparently cured by feeding butter. As this occurred in the course of the investigation of another problem, they did not attempt to repeat it. Although we have tried to produce this symptom in a considerable number of birds, only one has shown any evidence of soreness of the eyes. Whether this is due to a similarity to the rat in vitamine requirement, to a non-susceptibility to this disease, or to other difficulties which characterize the chicken as an experimental animal, we are not yet prepared to state.

One bird (no. 3), which was placed when half-grown upon the same commercial casein ration fed to rabbits and rats (using the same lot of casein) maintained itself for a period of 6 months with no apparent sign of malnutrition. During this time it consumed its feces constantly, a feature of its behavior which was shared by many of the rabbits on the same ration. In the case of the rabbits, however, this did not prevent rapid nutritive failure and death.

We have been hampered considerably in working with younger chicks with the development of leg-weakness, which appears to be a general symptom of malnutrition in chickens and which was not prevented by intimately mixing 10 per cent of paper pulp in the ration (8). This has led us to a study of the factors involved in the production of leg-weakness in chickens, which will be reported later. One of the chicks which was put on the casein ration at a weight of 345 grams developed, after 2 months on the ration, a very dull cornea, edematous eyelids, and a slight watery exudate which somewhat resembled xerophthalmia in the rabbit at an early stage. This bird is the only one which has shown any evidence whatsoever of this eye symptom. Chickens which were older when placed on this ration have corroborated the record of bird 3. Further data on the Vitamine A requirement of chickens will be reported in connection with our work on leg-weakness.

SUMMARY

Differences in the Vitamine A requirements of various species of animals are noted. It is definitely shown that the rabbit requires a larger allowance of this vitamine than the rat, pig and possibly the chicken.

Data are presented which show that rabbits are probably unable to grow successfully on a purified ration so supplemented as to be complete for the optimum nutrition of the rat.

Certain rations which quickly cause scurvy in a guinea pig and which were otherwise satisfactory produced optimum growth in the rabbit with no signs of scurvy.

A curious paralysis of the legs of rabbits was observed on certain cereal rations.

BIBLIOGRAPHY

(1) Nelson and Lamb: This Journal, 1920, li, 530.
(2) Osborne and Mendel: Journ. Biol. Chem., 1920, xli, 552.
(3) McCollum, Simmonds and Pitz: Journ. Biol. Chem., 1917, xxix, 341.
(4) Hart, Steenbock and Hoppert: Science, 1920, lii, 318.
(5) Parsons: Journ. Biol. Chem., 1920, xliv, 587.
(6) Steenbock and Boutwell: Journ. Biol. Chem., 1920, xli, 81.
(7) Wilkins and Dutcher: Journ. Amer. Vet. Med. Assoc., N. S., 1920, x, 666.
(8) Osborne and Mendel: Journ. Biol. Chem., 1918, xxxiii, 433.
Hart, Halpin and Steenbock: Ibid., 1920, xliii, 421.

THE REFLEX PRODUCED BY CHEMICAL STIMULATION OF THE DEEPER RESPIRATORY PASSAGES

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Contribution no. 87 from the Department of Anatomy Northwestern University Medical School

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The respiratory and circulatory reflexes produced by irritant gases applied to the mucous membrane of the nasal cavities were described in detail by Kratschmer (3), who observed that these differed from the effects produced by the action of the same gases in the deeper respiratory passages. The reaction produced by stimulation in the nostril he showed to be due to impulses passing through the trigeminal nerve. François-Franck (2) found the mucous membrane below the vocal cords to be entirely insensitive to stimulation by irritant vapors, according to Brodie and Russell (1). The latter investigators, however, obtained responses from all parts of the respiratory tract, the sensitivity decreasing from above downwards, but failed to contrast those from the upper and lower parts. Cutting the pulmonary branches of the vagi they found to destroy completely the effect of chloroform vapor in the lungs, and hence they conclude that the nerves excited are the alveolar branches of the vagus.

Roger (6) emphasized the contrast, the existence of which Kratschmer had indicated, between the effects of the application of irritant vapors to the mucous membrane in the upper and in the lower parts of the respiratory tract. Stimulation of the nasal mucous membrane of the rabbit he found to produce a sudden arrest of respiration with the lungs emptied, this being followed about 1 second later by a drop in blood pressure with an increase in the amplitude of the pulse beats. After about 9 seconds respiration is resumed rather slowly, and gradually increases in rate until the normal rhythm is restored, at about the same time as the blood pressure after a period of elevation following the primary depression also returns to normal. If the gas be led directly into the trachea, the response is one sudden, very deep *inspiration*, followed by a rather slow expiration, and then about 5 seconds apnea.

Respiration is then resumed and rapidly returns to normal. The circulatory effects are similar to those produced by stimulation in the nostril, but are less durable. Double vagotomy is found to have *no effect upon either of the respiratory reflexes.* The blood pressure, however, no longer drops, but rises markedly with only a slight instead of a great increase in the amplitude of the beats. These circulatory effects are the same whether the gas be applied in the nostril or through a tracheal cannula, so that it is evident that the changes in the circulatory response which Roger found to be produced by cutting the vagi must have been due to interruption of the efferent, cardio-inhibitory fibers.

Mayer, Magne et Plantefol (4) give a considerably different account of these reflexes, as seen in the rabbit and other animals. Application of the gas in the outer respiratory passages they find to cause a slowing or arrest of respiration. Passed directly into the trachea, so as to reach the bronchi and lungs, the gases produce a sharp *expiration*, followed by several jerky respirations. Then, inhalation continuing, respiration is very greatly accelerated, the polypnea produced by passing the vapor for 5 seconds often lasting as long as half an hour. Cutting both vagi *entirely prevents these phenomena*, or stops them if they are already present.

The main purpose of the present investigation was to determine whether or not the afferent paths concerned in these reflexes from the trachea, bronchi and lungs run through the vagus nerves, and whether the primary response of the respiratory mechanism is in the direction of inspiration or in that of expiration.

The problem was suggested by Dr. S. W. Ranson, to whom the writer is moreover greatly indebted for the hospitality of his laboratory and for an active interest in the experiments about to be reported.

Material and methods. All the experiments were carried out upon dogs. These were given an under-dose of morphine, anesthesia being completed first with chloroform, which was followed by urethane administered intravenously. A cannula was inserted so as to lead into the lower part of the trachea and was connected with a Y-tube, of which one arm led to a recording tambour, while the other was left open to allow respiration. A tube let into the side of the cannula provided for the application of the gas, which was accomplished by bubbling air under gentle pressure through a bottle of liquid ammonia or ether and then leading it through this tube into the cannula. The air was allowed to pass for 5 seconds. The blood pressure was recorded by con-

necting the carotid artery with a mercury manometer provided with a writer.

The following account is based upon the tracings obtained from twelve dogs, ten of which include the results of stimulation both before and after double vagotomy.

Observations. Of the two gases employed, ammonia produced considerably stronger stimulation than ether, the response to the former usually being markedly more vigorous than that to the latter. This is the opposite effect to that described by Roger, who diluted his ammonia with an equal quantity of water before bubbling the air through it, and then found it to be a much weaker stimulant than ether.

The application of ammonia or ether to the nasal mucous membrane produced a total arrest of respiration, not, however, with the lungs emptied, as described by Roger. This period of apnea is followed by slow, very deep respiration. The blood pressure was not found to drop below the level reached with each normal respiration, but when respiration is resumed the pressure rises considerably and the amplitude of the beats is increased. In fact, the vasomotor reflex in the normal dog corresponds with Roger's description of the response in the rabbit after cutting the vagi. In the present experiments the latter operation was not found to affect the reflex. While this is aside from the present subject, it is mentioned on account of the difference from Roger's description.

Attention was concentrated, however, upon the reflexes produced by blowing the gas into the trachea and bronchi. These were found to conform more nearly to the description of Mayer, Magne and Plantefol than to that of Roger. The appearance of the respiratory tracings obtained from different animals varied considerably, and examination of these seems to show that the response is made up of two elements, which may vary greatly in their relative importance. These two factors are forced expiration and an inhibition of inspiration. Probably the differences in the relation of these may be due to differences in the depth of anesthesia, and possibly in the strength of stimulation—perhaps also to fatigue in the mechanism, since successive applications of the stimulus often produced somewhat different tracings. There is a rather long latent period, which seems to vary with the amount of mucus in the respiratory passages.

The reaction commences almost invariably with an *expiratory* spasm, which may be interrupted by brief periods of apnea, producing a tracing made up of rising steps (fig. 1), or may at once empty the lungs more

or less completely. The sudden, vigorous expiratory contraction may be followed by a longer period of apnea, or of slight respiratory movements with the lungs practically empty (figs. 2*A*, 3*A*), or else by a gradual relaxation, often lasting a considerable length of time, which brings the tracing back to about the normal level (fig. 3*B*). There were cases, on the other hand, in which the inspiratory inhibition appeared to be almost entirely absent, the reflex response being a period of very rapid and deep breathing, such as is seen in figure 2*A* after the inhibitory effect has disappeared. Between these types is an intermediate one which resembles that represented in figure 1, but in which there is sufficient inspiratory movement to keep the tracing on a level instead of forming a series of steps.

The circulatory response consists of a marked, rather slow drop in blood pressure frequently, but by no means always, preceded by a brief rise. The pressure returns to normal usually a little more slowly than it fell.

Both the respiratory and the circulatory reflexes are absolutely unaffected by double vagotomy. In several cases the inspiratory inhibition seemed to be less marked after the operation, but in others it appeared more distinct, so that the character of the reflex was evidently entirely unchanged. Thus it is clear that despite the reports of Brodie and

Fig. 1. Results of projecting ammonia vapor through a tracheal cannula in a normal dog.

The upper tracing records respiration, a rising line representing expiration, a falling line inspiration. The middle tracing shows the blood pressure, and the lower one the time in seconds, the gap representing the period during which the vapor was passed into the cannula.

Russell and of Mayer, Magne and Plantefol, the afferent paths concerned do not run through the vagi.

A number of attempts were made to study the effect upon the reflexes of complete transection of the spinal cord in the lower cervical region, but unfortunately the animals all died within a short time after the

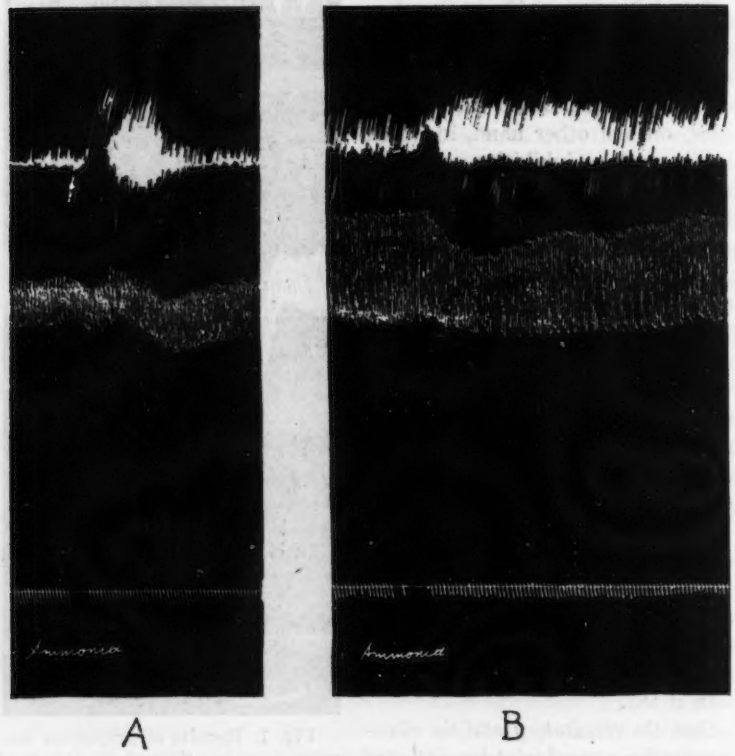

Fig. 2. Results of projecting ammonia vapor through a tracheal cannula: *A*, before and *B*, after double vagotomy. The tracings are arranged as in figure 1.

operation, and no trustworthy results were obtained. While this desirable confirmation is lacking, the above results clearly indicate that the main, if not the sole afferent paths concerned must run through the sympathetic to the spinal cord and up it to the respiratory and vasomotor centers.

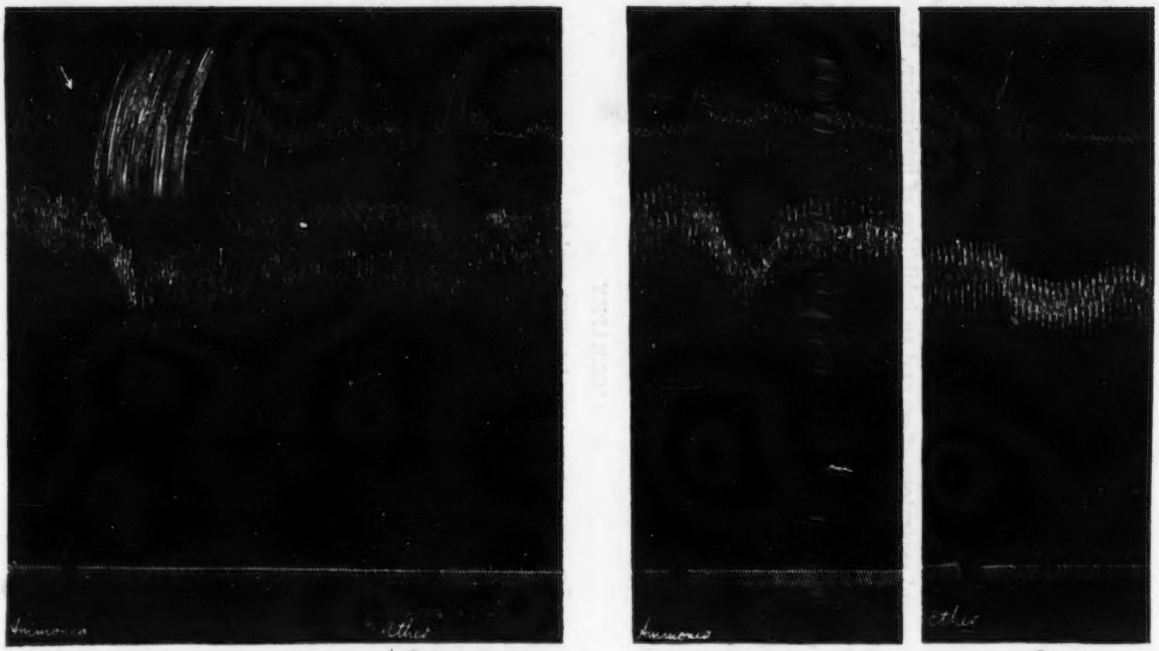

Fig. 3. Results of projecting ammonia vapor, *A*, *C*, and ether vapor, *B*, *D*, through a tracheal cannula. *A* and *B*, normal animal. *C* and *D*, after section of both vagi. Tracings arranged as in figure 1.

SUMMARY

The respiratory and circulatory reflexes aroused by projecting an irritant vapor into the trachea, bronchi and lungs have been stated by certain observers to be obliterated by double vagotomy and by others to be unaffected by this operation. The description of the respiratory response also varies considerably.

The respiratory reflex obtained in dogs by stimulation of the deeper respiratory passages with ammonia and with ether is found to consist of an increased expiratory effort and an inhibition of inspiration, these two elements appearing in varying proportions.

The vasomotor response consists of a rather gradual fall in blood pressure, followed at once by a somewhat slower return to normal. In some cases the fall is preceded by a brief rise in pressure of much smaller degree.

Both the respiratory and the circulatory reflexes are entirely unaffected by section of both vagi, and it is probable that the afferent impulses concerned pass through sympathetic branches to the spinal cord and up it to the medulla oblongata.

BIBLIOGRAPHY

(1) Brodie and Russell: Journ. Physiol., 1900, xxvi, 92.
(2) Francois-Franck[1]: Trav. du Labor. de Marey, 1876, ii, 221.
(3) Kratschmer: Sitzungsb. d. k. Akad. Wien., 1870, lxii, Abth. 2, 147.
(4) Mayer, Magne et Plantefol: Compt. Rend., 1920, clxx, 1347.
(5) Möllgaard: Skand. Arch. f. Physiol., 1912, xxvi, 315.
(6) Roger: La Presse Medicale, 1917, xxv, 73.

[1] This paper inaccessible to the author.

INFLUENCE OF GLANDS WITH INTERNAL SECRETIONS ON THE RESPIRATORY EXCHANGE

III. Effect of Suprarenal Insufficiency (by Removal) in Thyroidectomized Rabbits

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In a previous paper (1) it was shown that by removing or crippling the suprarenal glands in rabbits striking changes in the respiratory exchange resulted. These could be grouped under three headings as follows:

a. Rabbits that live indefinitely after removal or injury of the suprarenals with no appreciable alteration in the respiratory exchange.

b. Rabbits that show an increase in the respiratory exchange followed by a fall to or below normal, whether dying within 2 or 3 weeks or living on indefinitely.

c. Rabbits that show a fall in the metabolic rate, beginning within 48 hours after removal or injury of the glands and continuing to death.

The details of these groups in relation to the kind of injury and completeness of removal of the suprarenals are given in the following table.

TABLE 1

SUPARENAL GLANDS	GROUP I, NO RISE IN HEAT PRODUCTION	GROUP II, RISE IN HEAT PRODUCTION	GROUP III, FALL IN HEAT PRODUCTION
Frozen*	8	6	1
(a) Complete removal—no accessories found	1	9	1
(b) Complete removal—accessories found	1	8	2
(c) Incomplete removal	4	1	
Totals	14	24	4

* Two (nos. 224 and 231) excluded on account of infection.

Possible causes of the increased heat production were discussed. It was pointed out that trauma, infection and injury to nerves could be eliminated as important factors and that the simplest explanation of the phenomena observed with the facts at present available was that the increased heat production was in part due to increased thyroid activity brought about by the removal of a regulatory and inhibitory influence normally exercised by the suprarenals.

Inconclusive evidence was presented indicating that the underlying factor in the increased heat production was removal or insufficiency of the cortex rather than of the medulla.

The experiments included in this paper were undertaken to obtain further data on the question whether the thyroid, as previously suggested, was an important factor in the increased heat production following suprarenalectomy. For this purpose the following procedures were carried out on six rabbits. *1*, "Normal" heat production records (using the Haldane apparatus as described in a previous paper) were obtained over a period of 2 weeks; *2*, the thyroid glands were removed as completely as possible, leaving the external parathyroids with undisturbed blood supplies and the heat production measured for the next 23 days when, *3*, the suprarenals were removed and further measurements of the respiratory exchange continued until the animals either died or were sacrificed. The protocols follow:

Protocol I. Rabbit 266. Male—several skin wounds from recent fighting—poorly nourished.

1921.

May 31. Began metabolic studies. Weight 1827 grams.

June 8. Urea N. = 10.0; creatinin 1.2.[1]

June 13. Under ether removed most of thyroid gland, enlarged, hyperemic, histologically moderate hyperplasia. Urea N. = 11.4; creatinin 1.7.

June 29. Opened widely large subcutaneous abscess on back and rump. Thyroidectomy wound completely healed.

July 6. Weight 2076 grams. Under ether removed right and left suprarenal glands, both enlarged and together weigh 0.66 gram, containing 0.70 mgm. epinephrin.

July 7. Dull, slow respirations, diarrhea.

July 12. Losing weight rapidly, dull, but ate oats, cabbage and hay.

July 14. Opened several other small subcutaneous abscesses in ischial region, suprarenalectomy wounds healed.

July 20. Emaciation, asthenia, diarrhea with blood, died. Autopsy one hour after death. Weight 1665 grams. Markedly emaciated, large incompletely drained subcutaneous abscesses on lower back and ischial

[1] Figures for urea N and for creatinin indicate mgm. per 100 cc. blood.

region. No definite thyroid tissue found, small hyperemic area on anterior wall of trachea about 0.5 cm. below cricoid, saved for sections. Histological examination showed it to be thyroid. Moderate amount of fat in subcutaneous tissue of neck. Thymus area fatty, active thymic tissue present. Lungs normal; heart very flabby and dilated; liver slightly reduced in size, dark brown; kidneys normal in appearance; suprarenalectomy wounds clean and healed; right and left suprarenal glands completely removed, no accessories found; spleen normal; pancreas not hyperemic; moderate amount of perirenal fat; testes reduced in size; rectum and straight gut

TABLE 2

Rabbit 266

DATE	ROOM TEMPERATURE	WEIGHT OF RABBIT	O_2 IN GRAMS, 2 HOURS	CO_2 IN GRAMS, 2 HOURS	R. Q.	TOTAL CALORIES, 2 HOURS,	CALORIES PER KOM. PER HOUR	
1921		*grams*						
May 31	22.8	1827	2.885	3.845	97	10.11	2.77	
June 2	23.1	1827	2.790	3.570	93	9.69	2.65	
June 6	23.1	1806	2.680	3.700	100	9.51	2.63	
June 9	24.1	1823	2.720	3.665	98	9.56	2.62	
June 17	24.8	1915	2.115	2.835	98	7.40	1.93	June 13. Thyroidectomy
June 21	25.8	1969	1.955	2.590	96	6.86	1.74	
June 27	27.3	2047	2.240	3.030	99	7.84	1.92	
July 5	26.9	2076	2.165	2.940	99	7.61	1.83	
July 7	27.0	2008	2.300	2.600	82	7.79	1.94	July 6. R. and L. suprarenalectomy complete
July 10	28.0	1924	2.050	2.555	91	7.05	1.83	
July 12	26.8	1888	2.405	2.730	83	8.10	2.15	
July 14	27.2	1829	1.730	2.405	103	6.18	1.69	Figured on basis of 100 R.Q.
July 16	25.0	1792	2.100	2.525	87	7.22	2.01	
July 18	26.6	1678	1.380	1.480	78	4.61	1.37	

hyperemic, small hemorrhages in mucosa. Gastric mucosa intact. Death due to suprarenal insufficiency and extensive cutaneous abscesses from old bites and possibly somewhat delayed by thyroidectomy.

Protocol II. Rabbit 267. Male, well nourished.

1921.

May 31. Weight 2207 grams. Began metabolic studies.

June 3. Urea N = 13.1; creatinin 1.6.

June 13. Under ether removed most of thyroid gland. Urea N = 16.3; creatinin 1.7. Thyroid small, normal histologically.

July 6. Under ether removed right and left surarenal glands completely; together weigh 0.75 gram, containing 0.78 mgm. epinephrin.
July 12. Active, ate full ration, developing tendency to attack by biting and scratching when hand is put in cage.
July 15. Wounds healed, becoming vicious.
July 23. Getting weaker, not so active, eats sparingly of ration.

TABLE 3

Rabbit 267

DATE	ROOM TEMPERATURE	WEIGHT OF RABBIT	O_2 IN GRAMS, 2 HOURS	CO_2 IN GRAMS, 2 HOURS	R. Q.	TOTAL CALORIES, 2 HOURS	CALORIES PER KGM. PER HOUR	
1921		*grams*						
May 31	24.4	2207	3.030	3.720	89	10.45	2.37	
June 6	23.1	2222	2.965	3.620	87	10.35	2.33	
June 9	24.1	2243	3.010	3.705	90	10.32	2.30	
June 17	24.8	2269	2.605	3.160	88	8.96	1.97	June 13. Thyroidectomy.
June 22	26.0	2294	2.505	3.205	93	8.70	1.90	
June 27	27.3	2366	2.735	3.305	88	9.37	1.98	
July 5	26.9	2392	2.440	3.480	104	8.94	1.87	Figured on basis of 100 R.Q.
July 8	28.5	2298	2.245	2.710	88	7.68	1.67	July 6. R. and L. suprarenalectomy complete
July 10	28.0	2387	2.100	2.820	98	7.36	1.54	
July 12	26.0	2363	2.280	2.930	94	7.89	1.67	
July 14	27.2	2331	2.175	3.095	103	7.95	1.71	Figured on basis of 100 R.Q.
July 16	25.0	2318	2.270	3.215	105	8.26	1.78	Figured on basis of 100 R.Q.
July 18	26.6	2284	2.205	2.815	93	7.64	1.67	
July 20	25.0	2254	2.115	2.795	97	7.35	1.63	
July 22	25.8	2169	1.680	2.215	96	5.87	1.35	
July 25	28.7	2125	2.125	2.280	78	7.11	1.67	
July 28	29.2	2156	1.800	2.250	91	6.21	1.44	
August 1	23.0	2124	1.975	2.555	94	6.88	1.62	
August 4	21.9	2049	2.115	2.470	86	7.13	1.74	
August 6	24.2	2015	2.065	2.240	79	6.91	1.72	
August 8	24.6	1952	1.480	2.010	99	5.20	1.33	

July 29. Urea N = 24.5; creatinin 2.6.
July 30. Very dull, eats sparingly of oats and lettuce.
August 5. Urea N = 22.45; creatinin 2.5.
August 9. Very dull, weak, unable to hold head up; gave salt solution extract of two fresh rabbit's suprarenals intraperitoneally.
August 10. Has not eaten, progressively weaker and died at 6 p.m., autopsy at once, weight 1930 grams. No thyroid tissue found at site of thy-

roid lobes; both external parathyroids present and normal; thymus atrophic; moderate amount of fat at site; lungs normal; heart very flaccid and dilated; liver dark brown red, much reduced in size; both suprarenal glands completely removed, sites clean and healed, no accessories found, small clumps of yellowish material (suprarenal emulsion injected yesterday) in omentum; spleen small; pancreas not hyperemic; kidneys pale, no pitting; moderate amount of perirenal fat; testes atrophic; stomach and intestines nearly free of food; stomach contains about 10 cc. nearly clear secretion, no ulcers.

Protocol III. Rabbit 269. Male, well nourished.

1921.

May 31. Weight 2434 grams. Began metabolic studies.
June 6. Urea N = 15.4; creatinin 1.6.
June 13. Under ether removed most of thyroid gland, firmly attached to and high on larynx, not enlarged. Urea N = 17.3; creatinin 1.9.
June 28. Under ether removed right suprarenal gland completely, weight 0.15 gram. Urea N = 22.8; creatinin 1.9.
July 6. Under ether removed left suprarenal completely, weight 0.24 gram containing 0.19 mgm. epinphrin, scanty perirenal fat visible.
July 8. Eating well, active, soft stools.
July 16. Suprarenalectomy wounds healed, strong, active.
July 29. Urea N = 25.2; creatinin 2.1.
August 6. In excellent condition, gaining weight.
August 19. Urea N = 28.4; creatinin 2.35.
August 30. Hearty, vigorous.
September 2. Urea N = 25.2; creatinin 1.9.
September 7. Weight 2705 grams. General appearance normal, strong, active. Sacrificed to terminate experiment. Autopsy at once. Regenerated fragment of very vascular thyroid 2 mm. in diameter on right side at site of ligature. Otherwise complete removal. Parathyroids normal; thymus apparently completely replaced by fat; left lung normal, right shows few patches of consolidation. Heart dilated, flaccid. Liver slightly smaller than normal, somewhat paler and a little tougher on section than normal. Both kidneys are normal. Spleen dark red, normal in size. Pancreas hyperemic. Right and left suprarenals completely removed. Five very minute accessory cortical masses were found—three on left and two on right side. None of the masses were over 0.5 mm. in diameter. On the fat of the right epididymis are two brownish-yellow masses approximately 2 mm. in diameter resembling suprarenal cortex. This was verified by histological examination. Testes normal. No enlargement of lymph glands. Abundant fat in all depositories. Stomach filled with food. Mucosa normal. Intestinal tract normal throughout.

Protocol IV. Rabbit 270. Male, well nourished.

1921

May 31. Weight 2124 grams. Began metabolic studies.
June 6. Urea N = 11.6; creatinin 1.7.

TABLE 4

Rabbit 269

DATE	ROOM TEMPERATURE	WEIGHT OF RABBIT	O_2 IN GRAMS, 2 HOURS	CO_2 IN GRAMS, 2 HOURS	R. Q.	TOTAL CALORIES, 2 HOURS	CALORIES PER KGM. PER HOUR	
1921		*grams*						
May 31	24.4	2434	2.640	3.480	96	9.22	1.89	
June 2	23.9	2479	2.985	4.145	102	10.65	2.16	Figured on basis of 100 R.Q.
June 6	23.4	2482	3.235	4.180	94	11.26	2.27	
June 9	24.8	2500	3.300	4.315	95	11.53	2.31	
June 17	25.3	2531	2.930	3.480	86	10.04	1.98	June 13. Thyroidectomy
June 22	28.0	2643	2.810	3.560	92	9.75	1.85	
June 27	29.1	2721	2.680	3.730	102	9.58	1.76	Figured on basis of 100 R.Q.
July 5	26.4	2676	2.620	3.465	97	9.11	1.70	June 28. R. suprarenalectomy complete
July 8	28.5	2688	2.660	3.315	91	9.15	1.70	July 6. L. suprarenalectomy complete
July 10	28.8	2582	2.625	3.150	88	8.93	1.73	
July 12	26.0	2606	2.680	3.560	97	9.36	1.80	
July 14	27.2	2606	2.565	3.720	105	9.56	1.83	Figured on basis of 100 R.Q.
July 16	25.0	2624	3.300	4.265	94	11.49	2.19	
July 18	26.6	2620	2.695	3.690	100	9.48	1.81	
July 20	25.0	2655	2.935	4.050	100	10.40	1.96	
July 22	24.4	2674	2.890	3.940	99	10.20	1.91	
July 25	28.1	2678	2.680	3.530	96	9.35	1.75	
July 28	29.2	2714	2.810	3.510	91	9.69	1.79	
August 1	23.0	2718	3.005	3.870	94	10.42	1.92	
August 8	24.9	2735	2.835	3.740	96	9.91	1.81	
August 12	26.2	2788	2.635	3.380	93	9.18	1.65	
August 16	24.2	2788	2.900	3.715	93	10.09	1.81	
August 19	24.4	2725	2.940	3.655	90	10.18	1.87	
August 23	26.6	2755	3.095	3.760	88	10.66	1.93	
August 30	25.7	2752	2.850	3.470	88	9.84	1.79	
September 6	28.0	2712	2.770	3.320	87	9.50	1.75	

June 13. Under ether removed most of thyroid gland, not enlarged. Urea N = 14.0; creatinin 1.8.

July 6. Under ether removed right and left suprarenal glands completely and together weigh 0.47 gram, containing 0.52 mgm. epinephrin.

July 12. Active, ate heartily of oats, hay and lettuce.

July 18. Wounds healed and stitches removed.
July 26. Getting duller and weaker, eats very little.
July 29. Urea N = 27.3; creatinin 2.6.
July 30. Very dull, possibly has eaten some lettuce and oats.
August 1. Died this afternoon and autopsy at once. Small fragment of very hyperemic thyroid approximately 2 mm. in diameter on right side, near site of old stump ligature; thymus present, considerable thymic tissue visible; lungs normal; heart dilated and flaccid; liver markedly

TABLE 5

Rabbit 270

DATE	ROOM TEMPERATURE	WEIGHT OF RABBIT	O_2 IN GRAMS, 2 HOURS	CO_2 IN GRAMS, 2 HOURS	R. Q.	TOTAL CALORIES, 2 HOURS	CALORIES PER KGM. PER HOUR	
1921		*gm.*						
May 31	25.2	2124	2.670	3.560	97	9.36	2.21	
June 4	23.8	2256	3.285	4.275	95	11.42	2.53	
June 7	23.2	2260	3.090	4.115	97	10.82	2.39	
June 10	24.3	2305	3.400	3.840	82	11.50	2.50	
June 17	25.5	2372	2.905	3.535	88	10.02	2.11	June 13. Thyroidectomy
June 22	28.0	2474	2.915	3.665	92	10.03	2.03	
June 27	29.1	2560	2.905	3.780	94	10.18	1.99	
July 5	26.9	2531	2.985	4.080	99	10.56	2.09	
July 8	28.5	2472	2.740	3.300	88	9.36	1.89	July 6. R. and L. suprarenalectomy complete
July 10	28.8	2424	2.750	3.130	83	9.29	1.92	
July 12	26.2	2296	2.720	3.555	96	9.42	2.05	
July 14	27.2	2227	2.550	2.875	82	8.61	1.93	
July 16	26.9	2232	2.170	2.865	96	7.59	1.70	
July 18	26.6	2192	2.295	2.875	91	7.94	1.81	
July 20	25.0	2138	2.460	3.045	91	8.41	1.97	
July 22	24.4	2068	2.040	2.670	95	7.13	1.72	
July 25	28.1	2030	2.030	2.530	91	6.99	1.72	
July 28	29.2	1969	1.875	2.245	87	6.42	1.63	
August 1	23.0	1827	1.475	1.765	87	5.05	1.38	

reduced in size, dark brown red; kidneys small, dark red, surfaces smooth, no "pitting"; right and left suprarenals completely removed, no accessories found, there is a brownish subperitoneal discoloration over right psoas muscle for some distance below kidney, probably the remains of a slight extraperitoneal extravasation of blood at time of suprarenalectomy; spleen small; pancreas slightly hyperemic; stomach contains moderate amount of food, no ulcers; testes small; moderate amount of abdominal and subcutaneous fat.

Protocol V. Rabbit 271. Male, well nourished.

1921

May 31. Weight 1858 grams. Began metabolic studies.

June 6. Urea N = 21.0; creatinin 1.7.

June 13. Under ether removed most of thyroid gland, external parathyroids high and may have injured blood supply; thyroid enlarged, hyperemic and histologically moderate hyperplasia. Urea N = 12.5; creatinin 1.7.

TABLE 6

Rabbit 271

DATE	ROOM TEMPERATURE	WEIGHT OF RABBIT	O_2 IN GRAMS, 2 HOURS	CO_2 IN GRAMS, 2 HOURS	R. Q.	TOTAL CALORIES, 2 HOURS	CALORIES PER KGM. PER HOUR	
1921		gm.						
May 31	25.2	1858	2.145	3.035	103	7.80	2.10	Figured on basis of 100 R. Q.
June 3	22.0	1913	2.800	3.780	99	9.79	2.56	
June 7	23.2	1901	2.750	3.920	104	10.07	2.65	Figured on basis of 100 R.Q.
June 10	24.3	1925	2.780	3.785	100	9.72	2.53	
June 17	24.0	2017	2.480	3.440	101	8.84	2.19	June 13. Thyroidectomy Figured on basis of 100 R.Q.
June 21	28.9	2074	1.940	2.880	110	7.40	1.78	Figured on basis of 100 R.Q.
June 27	29.1	2103	2.370	2.960	91	8.17	1.94	June 28. R. suprarenalectomy complete
July 5	26.4	2081	2.245	2.910	95	7.77	1.87	
July 7	27.0	2056	2.340	2.530	79	7.81	1.90	July 6. L. suprarenalectomy complete
July 10	28.0	2038	2.055	2.500	89	7.03	1.72	
July 12	26.8	2045	2.250	2.785	90	7.76	1.90	
July 14	28.5	1986	2.085	2.585	91	7.14	1.80	
July 16	26.9	1975	1.565	2.230	104	5.73	1.45	Figured on basis of 100 R.Q.
July 18	27.0	1947	1.875	2.225	86	6.42	1.65	
July 20	27.0	1877	1.270	1.915	111	4.92	1.31	Figured on basis of 100 R.Q.
July 22	24.4	1849	1.345	1.730	94	4.66	1.26	
July 25	28.1	1778	1.280	1.460	83	4.33	1.22	

June 28. Under ether removed right suprarenal completely, weight 0.17 gram, containing 0.18 mgm. epinephrin. Urea N = 20.3; creatinin 1.8.

July 6. Under ether removed left suprarenal gland completely, weight 0.24 gram, containing 0.24 mgm. epinephrin.

July 7. Dull, not eating, diarrhea.

July 9. Ate heartily of oats, cabbage and hay, diarrhea.

July 15. Dull, but eating well.

July 19. Adrenalectomy wounds completely healed, getting weaker, eating very little.

July 24. Very dull, weak, soft stools, weight 1778 grams.

July 26. Died this afternoon and autopsy at once. No thyroid found, larynx saved for sections; thymus atrophic, but large mass of fat at site; lungs normal; heart dilated and flaccid; spleen small; right and left suprarenal glands completely removed, a possible accessory suprarenal on left renal vein; negative histologically; slight "pitting" of kidneys, the depressions being dark red in color; testes atrophic; abundant abdominal and subcutaneous fat; pancreas pale; stomach contains a fair amount of food, four small hemorrhages into mucosa of cardiac portion. Looks like typical suprarenal death.

Protocol VI. Rabbit 272. Male, well nourished.

1921

June 1. Weight 1930 grams. Began metabolic studies.

June 6. Urea N = 15.4; creatinin 1.6.

June 13. Under ether removed most of thyroid gland, situated very high on larynx, slightly enlarged. Urea N = 17.9; creatinin 1.7.

June 28. Under ether removed right suprarenal gland completely, weight 0.28 gram, containing 0.36 mgm. epinephrin. Urea N = 21.4; creatinin 1.8.

July 6. Under ether removed left suprarenal gland completely, weight 0.34 gram, containing 0.33 mgm. epinephrin. Abundant perirenal fat.

July 8. Eating well, very quiet.

July 16. Eats entire ration, quiet; suprarenalectomy wounds healed completely.

July 29. Urea N = 22.7; creatinin 1.9.

July 30. Looks normal, quiet, eats heartily.

July 19. Urea N. = 24.5.

September 1. Strong, healthy, normal-looking rabbit.

September 2. Urea N. = 25.2; creatinin 3.1.

September 7. Weight 2227 grams. Sacrificed to terminate experiment. Autopsy at once. There is a fragment of very vascular thyroid 1.5 × 2 mm. on right side attached to larynx and a very thin veil-like fragment on anterior surface of trachea approximately 1 × 2 mm. Parathyroids normal; thymus replaced by fat; both lungs normal; heart normal, muscle firm; liver normal in size and consistency; spleen small, pale; pancreas not hyperemic; right and left suprarenals completely removed, on the left side there is a small accessory suprarenal in the fat above and external to the kidney, also two other masses approximately 1 × 1 mm. along spermatic vein near pelvic rim. On the right side there is a large accessory suprarenal 1.5 mm. in diameter on the peritoneum external and above the kidney, also another accessory suprarenal 1 mm. in diameter on the anterior surface of renal vein at its junction with the inferior cava. In all five large accessory suprarenals were found; kidneys are smooth, normal in size and clor. There is an oval dark purplish encapsulated glandular mass roughly 3 mm. in length in the fat of each epididymis. They resemble accessory spleens in appearance but on histological examination were found to be extremely congested, hemorrhagic and hypertrophic accessory suprarenals. Stomach filled with food; mucosa normal as is also the intestine throughout; enormous deposits of fat in all depositories; bladder distended with clear urine.

TABLE 7

Rabbit 272

DATE	ROOM TEMPERATURE	WEIGHT OF RABBIT	O_2 IN GRAMS, 2 HOURS	CO_2 IN GRAMS, 2 HOURS	R. Q.	TOTAL CALORIES, 2 HOURS	CALORIES PER KGM. PER HOUR	
1921		gm.						
June 1	24.0	1930	2.890	3.470	87	9.92	2.57	
June 3	22.0	1972	3.010	3.610	87	10.32	2.62	
June 11	25.2	1981	3.070	3.840	91	10.60	2.68	
June 18	24.0	2060	2.455	3.245	96	8.60	2.09	June 13. Thyroidectomy
June 21	28.9	2099	2.345	3.030	96	8.03	1.91	
July 5	26.4	2134	2.500	3.070	90	8.55	2.00	June 28. R. suprarenalectomy complete
July 8	28.5	2121	2.100	2.735	89	7.69	1.81	July 6. L. suprarenalectomy complete
July 10	28.8	2158	2.355	3.240	100	8.32	1.93	
July 12	26.2	2150	2.425	3.360	101	8.63	2.01	Figured on basis of 100 R.Q.
July 14	28.5	2157	2.105	2.765	96	7.33	1.70	
July 16	26.9	2126	1.965	2.840	105	7.30	1.72	Figured on basis of 100 R.Q.
July 19	26.9	2145	2.270	3.055	98	7.97	1.86	
July 20	27.5	2146	2.040	2.895	103	7.44	1.73	Figured on basis of 100 R.Q.
July 22	24.4	2150	2.210	2.965	97	7.79	1.81	
July 25	28.1	2138	1.845	2.695	108	6.92	1.62	Figured on basis of 100 R.Q.
July 28	29.2	2167	2.155	2.955	100	7.59	1.75	
August 1	23.0	2168	1.860	2.845	113	7.31	1.69	Figured on basis of 100 R.Q.
August 8	24.6	2265	2.080	2.930	102	7.53	1.66	Figured on basis of 100 R.Q.
August 12	26.4	2286	2.300	2.945	93	7.99	1.75	
August 16	24.2	2244	2.050	2.805	101	7.21	1.61	Figured on basis of 100 R.Q.
August 19	24.4	2212	2.080	2.790	98	7.28	1.65	
August 23	23.0	2250	2.270	3.205	103	8.23	1.83	Figured on basis of 100 R.Q.
August 30	25.7	2309	2.225	2.950	97	7.76	1.68	
September 6	28.2	2232	2.270	2.530	82	7.58	1.70	Second 2 hours. Discarded first 2 hours on account of change of forceps.

Measurements of the respiratory exchange before thyroidectomy show the usual individual variations—the average per kilogram per hour being 2.13, 2.33, 2.41, 2.46, 2.62 and 2.67 calories for rabbits 269, 267, 270, 271, 272 and 266 respectively.

Following thyroidectomy the usual absolute decrease in the total heat production was obtained in five rabbits. It remained unchanged in one (270)[2] and the autopsy showed the thyroid had been incompletely removed. When the heat production was figured for calories per kilogram per hour a decrease was obtained in all experiments—being 31 per cent in rabbit 266, 20 per cent in rabbit 267, 20 per cent in rabbit 269, 13 per cent in rabbit 270, 24 per cent in rabbit 271 and 24 per cent in rabbit 272.

Following thyroidectomy gains in body weight varying from 200 to 400 grams and associated with rising respiratory quotients were observed. Twenty-three days after thyroidectomy the second suprarenal gland was removed (in three rabbits both suprarenals were removed at the same operation). Measurements of the respiratory exchange were continued on alternate days until the rabbit either died or further frequent measurements seemed unnecessary.

One rabbit died on the 14th, one on the 20th, one on the 26th, one on the 35th and two were sacrificed on the 63rd day after suprarenalectomy. The two rabbits that were sacrificed had apparently recovered and seemed normal in all respects except for the decreased heat production. Seven accessory suprarenals were found in each of these rabbits and there can be little doubt that their survival and recovery depended largely on this fact. No accessory suprarenals were found in the remaining four rabbits. The main suprarenal glands had been completely removed in all.

Thyroidectomy did not obviously modify the period of survival following suprarenalectomy. Loss of body weight likewise was as great as with

[2] The autopsy performed 49 days after thyroidectomy revealed a mass of vascular thyroid tissue 2 mm. in diameter on the right side at the site of the right lobe ligature. The thyroid was thought to have been completely removed and the present mass indicates regeneration. In two others, examined 80 days after thyroidectomy, considerable thyroid tissue was found. It is difficult to remove completely the thyroid in the average rabbit even after an enormous experience, the reason being that the anterior and superior border of the lobes are frequently firmly attached to the lower border of the larynx and in separating these attachments, fragments—possibly only a few cells—may be left, and on account of their great capacity for survival and growth appreciable masses often regenerate within a month.

suprarenalectomy alone although there has been more visible fat in the usual depositories. This probably is the result of the larger fat reserves in thyroidectomized rabbits. No thyroid tissue was found post-mortem (as determined both macroscopically and microscopically) in two, while in three regenerated masses of very vascular thyroid were present. In rabbit 266 a few thyroid follicles were found histologically.

The observation of particular importance in these experiments was the *absence* of a significant rise in heat production following suprarenalectomy in five of the six experiments (see tables 2 to 7). In suprarenalectomized rabbits with intact thyroids a rise of over 10 per cent was obtained in seventeen out of a total of twenty-two or 77 per cent. In no instance did the heat production after suprarenalectomy rise to the normal control level and in only one instance (rabbit 269) did it rise appreciably above the lowest level after thyroidectomy. In this instance the rise in heat production was typical. It began on the 4th day, reached its maximum on the 10th day following suprarenalectomy then gradually fell during the following 2 weeks but never to the low level reached following thyroidectomy alone. The animal recovered apparently completely except for the lowered heat production. When sacrificed seven small accessory suprarenals together with a hyperplastic thyroid fragment 2 mm. in diameter were found. In another instance with recovery (rabbit 272) that did not show a post-suprarenalectomy rise in heat production, seven very large accessory suprarenals together with two hyperplastic fragments of thyroid were found when sacrificed. We believe the absence of the rise in heat production in this rabbit was dependent upon the large amount of accessory suprarenal cortex. These observations further illustrate the very small amount of functionally active suprarenal cortex necessary for the maintenance of health and how narrow the limits are even anatomically within which one must work in order to obtain a post-suprarenalectomy rise in heat production and survival of the animal. Also these experiments illustrate what small amounts of thyroid tissue under favorable conditions of nutrition may suffice to notably influence the heat production. Thus rabbit 270 showed only a very slight reduction in heat production following what was believed to be a complete thyroidectomy but the autopsy showed a very hyperplastic fragment of thyroid approximately 2 mm. in diameter. A comparison of the metabolism tables shows that the heat production in the three (269, 270 and 272) rabbits in which thyroid was found at autopsy was maintained on a much higher plane than in the two (267 and 271) where no thyroid

was found at autopsy. The few thyroid follicles found on microscopical examination in rabbit 266 probably had no measurable effect on its metabolism and therefore it should be grouped with rabbits 267 and 271.

Blood urea and creatinin were determined at intervals throughout the experiments. The data are given in the protocols.

Following suprarenalectomy the creatinin content of the blood showed a significant rise. The possible slight rise in the urea following thyroidectomy might be mentioned. These data could not be correlated with any gross renal lesions.

DISCUSSION

Partial destruction of the function of the suprarenal cortex in rabbits usually (77 per cent of our series) causes a rise in heat production and removal of the thyroid gland in our experiments has prevented it. These facts if confirmed would establish another thyroid-suprarenal interrelationship which, with the facts at present available, seems to depend on an antagonistic action of the thyroid and suprarenal cortex. The much discussed interrelationship of previous observers was assumed to depend on the mutual stimulating effects of the thyroid and chromaphil tissues. Thus, the original schema of Eppinger, Falta and Rudinger (2) assumed that the thyroid and the chromaphil tissues mutually aided each other. Asher and Flack (3) were the first to produce experimental evidence in support of this hypothesis by showing that a given dose of adrenalin produced a greater pressor effect after thyroid stimulation than before such stimulation or following removal of the thyroids. Levy (4) confirmed these observations. The so-called Asher-Goetsch (5) adrenalin sensitization test is a clinical diagnostic application of Asher and Flack's original observations. Later work by Peabody, Wearn, Clough, Tompkins and Sturgis (6), by Tompkins, Sturgis and Wearn (7), by Sandiford (8) in man and by Marine and Lenhart (9) in rabbits, has demonstrated that the thyroid is not necessary for this reaction although it seems established that a hyperfunctioning thyroid or even an intact normal gland increases the reaction. The work of Dreyer (10), Elliott (11) and later of Stewart and Rogoff (12) using more technical methods has shown that section of the nerves to the suprarenals reduces the epinephrin output below the level of detectability as determined by a very sensitive biological test without any apparent injury to the animal's health. This also indicates that thyroid stimulation by epinephrin is at least relatively unimportant. Since heat production

sufficient to maintain vegetative life can go on probably for months after complete removal of the thyroid and since adrenalin in excessive doses can stimulate heat production in the absence of the thyroid, it follows that there are many other more basic factors than either the thyroid or the suprarenal medulla involved in the maintenance of heat production. It is possible, however, that the thyroid gland and the chromaphil tissues through stimulation, and the thyroid gland and suprarenal cortex through antagonistic actions, may provide a means for maintaining a higher level of and for bringing about greater variations in heat production. Our observations that suprarenalectomy with intact thyroids causes a rise in the respiratory exchange and that thyroid removal prevents or greatly lessens this rise, if confirmed, would indicate that the rise is dependent upon an increased activity of the thyroid and that it is independent of any stimulating substance produced either in the cortex or medulla. The evidence further suggests that the suprarenal gland, and especially its cortex, exercises a regulatory, restraining or inhibitory control over the thyroid activity and the removal of this cortical influence acts as a much more powerful stimulus to thyroid activity than epinephrin.

This conception of thyroid control would explain the hyper-susceptibility of certain individuals to adrenalin as in part dependent upon a primary insufficiency of the suprarenal cortex and a thyroid-hyperactivity secondary to the cortical insufficiency. Similarly, the hypersusceptibility to desiccated thyroid or thyroxin could be explained as in part dependent upon cortical insufficiency and normal or increased activity of the medulla. Likewise, the observations of R. G. Hoskins (13), E. R. Hoskins (14), Herring (15) and others that thyroid feeding causes enlargement of the suprarenal cortex and increased epinephrin store in the medulla; and of McCarrison (16) that inanition also causes enlargement of the suprarenal cortex and increased epinephrin store could be interpreted as attempts to depress thyroid function and tissue oxidation. Students of thyroid physiology have contended that the fundamental manifestations of exophthalmic goiter could not be explained on a thyroid hypothesis alone and that the alterations in thyroid structure and the increased thyroid function were secondary to and symptomatic of more important lesions elsewhere (17), (18). If the suprarenal cortex directly or indirectly exercises a restraining, regulatory or inhibitory control over the thyroid gland it will have introduced a conception of exophthalmic goiter which though differing widely from the still popular conception offers a possible explanation for many of its fundamental

manifestations. This conception would assume a weakness or exhaustion of the cortical function with unchanged, decreased or even increased medulla function as important underlying causes of the increased thyroid activity.

The possible importance of cortical insufficiency as a factor in the etiology of thyroid hypertrophies and hyperplasias of puberty, pregnancy, menstruation and menopause might also be suggested. One of the greatest arguments against a direct infectious theory of the origin of simple goiter has been the fact that beginning with the age of puberty human thyroid hypertrophy and hyperplasia is six or eight times more frequent in the female while in the lower animals or below puberty in man sex has no detectable influence on the incidence. It is known that bacterial toxines cause thyroid hyperplasia (19) and it is possible that this is an indirect action through the suprarenal cortex.

As to the means by which the suprarenal cortex normally exercises a control over thyroid activity only speculation is possible at present. Nothing definitely is known of the chemical activities of the cortex. It is possible that it elaborates some specific substance which regulates thyroid activity directly or which operates indirectly through control of the general tissue oxidations. In this connection one naturally turns to the "lipoids" which make up so large a part of the cortex and which many believe play an important rôle in the oxidation processes going on in all tissues. Lastly, while the thyroid-suprarenal cortex interrelationship has been emphasized in this paper, it is not believed that it is an isolated or independent one. On the contrary, there is abundant evidence that these tissues' functions must be intimately related directly or indirectly to many, probably all, other tissue functions both as regards accelerator and inhibitory actions and as regards simpler and more complex interrelations. We refer particularly to the suprarenal medulla, the interstitial cells of the sex glands, the thymus and lymphoid tissues, the hypophysis, the liver and the pancreas,

CONCLUSIONS

1. Partial but sufficient destruction of the function of the suprarenal cortex in rabbits with intact thyroids usually leads to an increased heat production.

2. Removal of the thyroid prevents or greatly lessens the increased heat production which usually follows partial destruction of the suprarenal cortex.

3. While epinephrin in large amounts may stimulate the thyroid, it is believed that exhaustion or insufficiency of the regulatory, inhibitory and restraining effect of the cortical influence is a much more important cause of increased thyroid activity and increased tissue oxidations in rabbits.

4. Many other tissues and more complicated glandular interrelations may be involved—especially that closely related tissue, the interstitial cells of the sex glands.

5. The observations included in this paper may have an important bearing on the etiology of both exophthalmic goiter and simple goiter of puberty, pregnancy, menstruation and menopause.

Note: The respiratory exchange measurements were made by Miss Anna Cipra and Miss B. L. Lowe, to whom we wish to express our thanks.

BIBLIOGRAPHY

(1) Marine and Baumann: This Journal, 1921, lvii, 135.
(2) Eppinger, Falta and Rudinger: Zeitschr. f. klin. Med., 1908, lxvi, 1.
(3) Asher and Flack: Zeitschr f. Biol., 1911, lv, 83.
(4) Levy: This Journal, 1916, xli, 492.
(5) Goetsch: Med. Rec., 1918, xciv, 567.
(6) Peabody, Clough, Sturgis, Wearn and Tompkins: Journ. Amer. Med. Assoc., 1918, lxxi, 1912.
(7) Tompkins, Sturgis and Wearn: Arch. Int. Med., 1919, xxiv, 269.
(8) Sandiford: This Journal, 1920, li, 407
(9) Marine and Lenhart: This Journal, 1920, liv, 248.
(10) Dreyer: This Journal, 1899, ii, 203.
(11) Elliott: Journ. Physiol., 1912, xliv, 374.
(12) Stewart and Rogoff: Journ. Pharm. Exper. Therap., 1917, x, 1.
(13) Hoskins: Journ. Amer. Med. Assoc., 1910, lv, 1724.
(14) Hoskins: Journ. Exper. Zoöl., 1916, xxi, 295.
(15) Herring: Quart. Journ. Exper. Physiol., 1917, xi, 47.
(16) McCarrison: Indian Med. Journ., 1919, vi, 275.
(17) Marine and Lenhart: Arch. Int. Med., 1911, viii, 265.
(18) Marine: Ohio State Med. Journ., 1920, xvi, 735.
(19) McCarrison: Quart. Journ. Med., 1909, xi, 279.

FURTHER STUDIES OF PARADOXICAL PUPIL DILATATION FOLLOWING LESIONS OF THE AFFERENT PATHS

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After section of one brachial plexus.[1] Through an interscapular incision the nerve trunks of the left brachial plexus were divided in cat 1. Typical paradoxical effects were elicited in the left pupil by adrenalin on the 7th, 11th and 23rd days. On the 75th day, after adrenalin had failed to elicit paradoxical effects, the scar region of the left brachial plexus was cut across. Seven days later adrenalin evoked paradoxical effects in the left pupil with typical reversal phenomena.

Protocol. Cat 1. Female, adult

DAY	TIME	REMARKS	PUPILS		MEMBRANES	
			Right	Left	Right	Left
			mm.	*mm.*	*mm.*	*mm.*
		August 12, 1919. Transected the nerve trunks of left brachial plexus				
	p.m.					
7th	12:50	Before adrenalin	3.0	2.5	5.0	5.0
	12:50½	Adrenalin m. ii. Paradoxical effects in left pupil	12.0	14.0	0.0	0.0
11th and 23rd		Paradoxical effects in left pupil				
75th		Paradoxical effects absent in left pupil. Cut across the scar region of the left brachial plexus				
	a.m.					
82nd	11:13	Before adrenalin	2.5	1.5	3.0	2.5
	11:13½	Adrenalin m. ii.	11.0	13.0	0.0	0.0
	11:14	In constriction. Paradoxical effects in left pupil with reversal phenomena	8.5	10.0	1.0	2.0

[1] For method of study, etc., see previous paper (1).

In cat 2 the left brachial plexus was similarly divided. Paradoxical effects were elicited in the left eye by adrenalin on the 7th and 14th days. After death on the 20th day the left was the larger pupil.

In another cat after section of the left brachial plexus paradoxical effects were evoked by adrenalin on the 7th, 12th and 23rd days. On the 25th and 31st days adrenalin instilled into the conjunctival sacs failed to cause appreciable dilatation in either pupil.

After section of structures about one shoulder without injury of the brachial plexus. In cat 3 the structures about the left shoulder were divided. The operation was identical with that performed on cats 1 and 2 but the brachial plexus was merely exposed and not otherwise interfered with. Adrenalin evoked typical paradoxical effects in the left pupil on the 8th day but failed to do so on the 3rd, 14th, 25th and 28th days. On the 28th day the structures about the left shoulder were again divided. Following this operation adrenalin evoked typical paradoxical effects in the left eye on the 7th, 11th and 12th days but failed to do so on the 4th. On the 14th day paradoxical effects were absent in the left eye before and after section of both vago-sympathetic nerves and in death.

After section of structures about both shoulders and crushing of one brachial plexus. In cat 4 (pregnant) the structures about both shoulders were divided as in exposing the brachial plexus; the nerve trunks of the right brachial plexus were then crushed with an artery clamp. Adrenalin evoked well-marked paradoxical effects in the right eye on the 8th, 11th and 18th days but failed to do so on the 3rd, 4th and 5th days. After the 18th day adrenalin no longer evoked unequivocal paradoxical effects in the right eye. On the 44th day after adrenalin had failed to elicit paradoxical effects in the right eye, the right brachial plexus was again crushed. Following this operation paradoxical effects were elicited on the 31st, 37th, 42nd and 44th days but could not be elicited on the 2nd, 3rd and 46th days. At the site of the skin incision a superficial ulcer formed about the 30th day. After suitable dressing healthy granulations appeared in a few days. On the 46th day the spinal cord was transected at root CV. On the 47th day adrenalin failed to elicit true paradoxical effects before and after division of both vago-sympathetic nerves and in death.

After injections of 60 per cent alcohol into the brachial plexus. Through an interscapular incision the trunks of the left brachial plexus were exposed and injected with 60 per cent alcohol in cat 5. On the 7th and 29th days, paradoxical effects were elicited in the left pupil. On the

13th day adrenalin instilled into the conjunctival sacs failed to elicit paradoxical effects. After the 29th day paradoxical effects could no longer be elicited in the left pupil by adrenalin.

In cat 6 the left brachial plexus was also injected with 60 per cent alcohol by open operation. Paradoxical effects were elicited in the left pupil by adrenalin on the 7th, 11th and 28th days. On the 19th day paradoxical effects could not be evoked in the left pupil. Unusually cold weather prevailed between the 20th and 27th days. On the 28th day paradoxical effects were again elicited in the left eye by adrenalin (fig. 1).

Protocol. Cat 6. Female, adult, non-pregnant

DAY	TIME	REMARKS	PUPILS		MEMBRANES	
			Right	Left	Right	Left
			mm.	*mm.*	*mm.*	*mm.*
		December 7, 1919. Injected left brachial plexus with 60 per cent alcohol				
	p.m.					
7th	12:15	Before adrenalin	1.0	1.0	3.0	3.0
	12:15½	Adrenalin m. ii. Paradoxical effects in left eye	12.0	13.0	0.0	0.0
11th	11:24	Before adrenalin	1.0	1.5	5.0	5.0
	11:24½	Adrenalin m. ii. Paradoxical effects in left eye	6.0	8.0	0.0	0.0
19th	12:30	Adrenalin m. ii. Paradoxical effects absent in left eye	11.0	9.0	0.0	0.0
		Zero weather prevailed from 19th to 27th day				
	a.m.					
28th	11:20	Before adrenalin	1.0	1.0	5.0	5.0
	11:20½	Adrenalin m. ii. Paradoxical effects in left eye (photograph)	11.0	13.0	0.0	0.0

After unilateral section or injury of the lower cervical nerve roots. In cat 7 the right posterior spinal nerve roots CV and VI were partially cut across. On the 10th day paradoxical effects were elicited in the right eye by adrenalin on the first trial but in two subsequent tests the left was the more sensitive pupil to adrenalin. On the 14th day the first test with adrenalin showed the left to be the more sensitive pupil but a later test caused greater dilatation in the right pupil. On and after the 24th day adrenalin no longer evoked paradoxical effects in the right eye.

In another animal the right posterior nerve roots C VI and VII were divided within the spinal canal. The animal died on the 9th day, the right pupil in death being considerably larger than the left.

After unilateral section or injury of the upper cervical posterior nerve roots. In cat 8 the right posterior nerve root C II was divided within the spinal canal. Well-marked paradoxical effects were elicited in the right pupil on the 7th and 14th days by ether and by adrenalin. The membrane showed only a mere trace of paradoxical withdrawal although the paradoxical dilatation of the pupil was marked. On and after the 21st day paradoxical effects could no longer be elicited in the right eye by adrenalin.

Fig. 1. Paradoxical effects in left eye on 28th day after injection of left brachial plexus with 60 per cent alcohol.

In cat 9 the right nerve root C I was evulsed without opening the spinal canal. Paradoxical effects were elicited in the right eye on the 7th, 21st (waning) and 28th (marked) but were absent on the 11th and 14th days. Extremely cold weather prevailed from about the 20th to the 28th days. On the 56th day paradoxical effects were evoked by adrenalin and by death.

After crushing nerve trunks C II III IV outside the spinal canal. In cat 10 nerves C II III and IV of the right side were exposed and crushed in an artery clamp a short distance from the dorsal root ganglia. The spinal canal was not opened and the related dorsal root ganglia were uninjured. Adrenalin evoked paradoxical effects in the right eye

on the 10th and 17th days but failed to do so on the 14th, 21st and 25th days.

In cat 11 the posterior divisions of nerves C II and III were crushed on the right side. Adrenalin failed to elicit paradoxical effects in the right eye on the 7th, 9th, 11th, 12th, 14th and 15th days. Well-marked paradoxical effects were elicited on the 17th and 18th days. On the 24th day feeble paradoxical effects were elicited but after this date hypersensibility to adrenalin was no longer present in the right eye. From the 25th to the 85th day adrenalin failed to elicit paradoxical effects in the right eye.

In cat 12 the posterior divisions of nerves C II and III in addition to nerve C I were crushed on the left side near their exit from the vertebral canal. On the 9th day paradoxical effects were evoked in the left eye by deep anesthesia but not by adrenalin. On the 11th and 12th days adrenalin failed to evoke frank paradoxical effects in the left eye. On the 13th day adrenalin evoked well-marked paradoxical effects in the left eye before and after section of both vago-sympathetic nerves. In death the left was the larger of the two pupils.

DISCUSSION AND SUMMARY

After section, alcoholic injection, or crushing of the brachial plexus, the period during which the paradoxical effects may be evoked by adrenalin, etc., corresponds closely with the paradoxical period observed after section, etc., of the sciatic nerve. The functional relationship between the afferent paths in the brachial plexus and the lateral grey columns of the upper thoracic segments (lower cilio-spinal center of Budge) is mainly uncrossed. This seems to be the determining factor not only of the incidence of the paradoxical phenomena in the homolateral eye but also of the shorter incubation period (averaging about 8 days) found after brachial plexus lesions as contrasted with an average of 10 to 12 days found after lesions of the sciatic nerve.

In cat 3 in which the brachial plexus was merely exposed, the plexus itself remaining untouched, the findings raised the question as to whether the paradoxical effects observed after lesions of the brachial plexus might not be wholly or in great part due to severence of numerous afferent paths other than those directly involved in the brachial plexus lesions proper. This question was satisfactorily answered by the findings in cat 4 in which both brachial plexuses were exposed and only one of them subjected to experimental injury. The appearance of true

paradoxical effects and their persistence for a period much longer than in cat 3, in which the brachial plexus was merely exposed, indicated that in lesions of the brachial plexus the paradoxical effects were mainly the result of the brachial plexus lesion proper though in part perhaps attributable to other afferent path lesions incidental to the operation.

The experiment on cat 8 in which the upper cervical roots were divided within the spinal canal also raised a question as to the possibility of the paradoxical pupillary phenomena being the result of unintentional damage to the spinal cord. This question was satisfactorily answered in the negative by the findings in cats 10, 11 and 12 in which the posterior division of spinal nerves C II and III were traumatized by crushing without interference with the spinal canal or its contents. These latter findings further emphasized the intimate functional relationship that exists between the pupil dilator mechanism and every portion of the periphery supplied by the spinal sensory nerves. This relationship has important clinical significance since in injuries about the shoulder involving the brachial plexus inequality of the pupils is usually interpreted to mean injury of the cervical sympathetic nerve or of the ciliospinal center of Budge. Compare the Klumpke-Déjérine sign. In view of the fact that injury of any sensory nerve trunk may cause well-marked inequality of the pupils, such an interpretation should be made only after rigid exclusion of lesions of the peripheral nerves. The effect of cold weather in re-inducing paradoxical phenomena after they had disappeared or begun to wane is seen in cats 6 and 9. The mechanism here consists of retrograde influence of cold upon the newly regenerating neurones in the dorsal root ganglia.

The absence of paradoxical effects in cat 6 on the 19th day and in cats 3, 7 and 8 on the 25th, 21st and 24th days respectively as well as the marked waning of them on the 21st day in cat 9 point to the time of disappearance of the paradoxical condition as closely coinciding with that observed after sciatic section. The conclusion therefore seems warranted that the paradoxical phenomena observed after brachial plexus section or injury are identical in kind and mechanism with those observed after section or injury of one sciatic nerve which in turn have been shown to be identical with the paradoxical phenomena observed after removal of one superior cervical ganglion or section of one cervical sympathetic nerve. The reappearance of the paradoxical condition 7 days after section on the 75th day of the scar regions of the divided brachial plexus in cat 1 further confirms what had been concluded in the case of sciatic section, viz., that the appearance and

disappearance of the paradoxical phenomena "are conditioned respectively by the suspension and restoration of specific neural functioning in the related neurone bodies of the dorsal root ganglia" (1).

CONCLUSIONS

1. After section or injury of one brachial plexus paradoxical pupillary phenomena may be elicited in the homolateral eye after a latent or incubation period which averages about 8 days.

2. After injury of one brachial plexus the paradoxical pupillary phenomena are identical in nature and mechanism with the phenomena observed after section or injury of one sciatic nerve or section, etc., of the cervical sympathetic nerve.

3. Moderate injury, such as crushing of any of the afferent spinal nerve bundles, as well as extensive injury of tissue without apparent injury of visible nerve bundles, is followed by a typical paradoxical period of shorter duration than the paradoxical period which supervenes after section or severe injury of one brachial plexus or one sciatic nerve.

4. The functional relationship between the periphery and the pupil dilator mechanism through the sensory nerves is such that tissue injury of all sorts or degrees may cause inequality of the pupil.

5. Care should be taken when interpreting clinically the significance of unequal pupils to give due consideration to possible injuries of the afferent paths in the peripheral nerves.

BIBLIOGRAPHY

(1) Byrne: This Journal, 1921, lvi, 113.

ENERGY EXPENDITURE IN SEWING

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For a number of years the work of the Office of Home Economics has included the use of the respiration calorimeter for investigations of the energy required in the performance of household tasks. Earlier experiments on a variety of household operations carried out by a normal, healthy woman have already been reported by the authors.[1] The present article gives the results of a special study of hand and machine sewing.

The respiration calorimeter is an apparatus by means of which the transformations of energy taking place in the human body may be measured simultaneously in two ways; one, the direct measurement of the heat eliminated by the subject, and the other, computations from the measurements of the oxygen consumed and carbon dioxid given off in respiration. Briefly stated, this instrument consists of a chamber 75 cm. × 120 cm. × 200 cm. and fitted with air-tight walls. There is a ventilating system by means of which air of known composition is continuously passed through the chamber and which is connected with an absorber system for measuring and removing the respiratory products. Other devices make it possible to carry away and measure the heat produced within the chamber. In the experiments here reported both methods of measuring the energy output were used, but as the direct measurements are fundamentally more accurate and as the computations from the respiratory data gave results practically identical with those obtained directly, the following discussion is based mainly upon the latter.

The present series consisted of 43 experiments made with the same subject, under conditions that were kept as uniform as possible, except as regards bodily activity, which was varied according to the nature of the individual experiments. In order to determine the energy required for a specific task the energy expenditure during the period of such

[1] This Journal, 1920, lii, 400.

work was compared with the expenditure during a period of complete rest and the difference between the two was taken to represent the energy required for the task itself.

The subject was a woman 28 years old, blond, 5 feet 6 inches tall, with an average weight of 139 pounds (63 kgm.) in uniform. The uniform consisted of a heavy cotton "middy blouse" and skirt and very light underclothing. Her health was, in general, excellent, but about six weeks after the beginning of these tests she had an attack of influenza which lasted for three weeks. When she returned to the laboratory she seemed to have recovered completely and her weight, which had fallen off four or five pounds during her illness, had returned to normal.

In order to secure uniformity in the energy required for digestive processes, breakfast was eaten at 7:45 a.m. on each experimental day and consisted always of the following: One-half grapefruit with 2 teaspoons sugar; 6 tablespoons flaked and toasted corn breakfast food with 2 teaspoons sugar and ½ gill cream; 3 slices buttered toast; and 1½ cup black coffee. No special record was kept of the other meals, but the subject tried to keep her diet reasonably uniform in kind and quantity.

The subject entered the calorimeter chamber at 10 a.m. The experimental measurements were begun between 11:00 and 11:30 a.m. and continued for two hours. During that time she followed carefully the prescribed routine in which she had been thoroughly drilled in advance. Her weight was taken at the close of each experimental period and, save for the change during her illness, remained practically constant throughout the five months covered by the test.

The results of all the experiments are summarized in table 1.

Rest experiments. Fourteen experiments were made to measure the energy expenditure of the subject when external muscular work was reduced to a minimum, seven of these preceding the attack of influenza and seven following it. Throughout each experimental period the subject rested in a bentwood chair, relaxing as much as possible and making no movements except those absolutely necessary. The average results of the first seven rest experiments served as a basis of comparison with the work experiments which preceded the illness of the subject, while the average of the seven later ones was used with those following her absence. It may be mentioned that there was only slight variation between the results of the individual experiments in any of the groups of the series. As is shown by table 1, the average energy expenditure

of this subject at rest was 66.1 calories per hour before her illness and 63.6 calories after it. This corresponds to 1.03 calories per kilogram of body weight.

The drop in energy expenditure after the attack of influenza is interesting as a confirmation of the theory that the depressant influence which such an infection exerts on the general metabolism continues after its more conspicuous effects have disappeared.

TABLE 1

Average energy expenditures of subject during rest and sewing

CHARACTER OF MUSCULAR ACTIVITY	NUMBER OF EXPERIMENTS IN AVERAGE	AMOUNT OF SEWING ACCOMPLISHED PER HOUR	ENERGY EXPENDED		
			Per hour		Per meter of sewing
			Total	For work alone	
		meters	*calories*	*calories*	*calories*
Rest	7		66.1		
Hand hemming					
Handkerchiefs	8	2.4	71.8	5.8	2.40
Sheets	3	3.4	71.8	5.7	1.70
Rest	7		63.6		
Hand hemming					
Sheets	5	3.4	69.1	5.5	1.70
Duck	5		69.2	5.6	
Blanket, 18 stitches per minute	2	3.4	67.9	4.3	1.25
Blanket, 30 stitches per minute	3		76.8	7.2	
Machine sewing, sheets					
Foot-driven machine	3	47.8	97.5	33.6	0.70
Motor-driven machine	2	55.9	72.4	8.3	0.15

Experiments with hand sewing. Previous work with hand sewing had suggested that the weight, size and texture of the material to be sewed might have some effect on the energy expended, as might also the speed at which the work was done. Special experiments were therefore planned to test these points. In order to eliminate as far as possible such variations as might be caused by using different kinds of stitches and by motions other than the actual sewing, only hemming stitches were used in the experiments, the hems were turned and basted in advance, and a supply of sewing threads 37 inches long was cut from the spool and placed where it could be reached with very slight motion.

The entire unused length of thread in the needle was pulled through at each stitch.

Throughout the two hours of each experiment the subject sewed steadily, seated in a bentwood chair and avoiding all movements except those of the sewing itself. The work was timed to the beat of a metronome so that an exact count could be made of the number of movements and a complete time-motion study secured. For all but one experiment the rate was 18 stitches to the minute, a speed chosen as one at which good sewing could be done without discomfort.

There were six tests with hemming on light linen handkerchiefs, 30 cm. square and weighing 3 grams each; eight tests with heavy starched cotton sheeting, 228 cm. square and weighing 850 grams; five tests with 8-ounce unbleached duck in strips 30 cm. long and weighing 200 grams; and two with army blankets 208 cm. square and weighing 1700 grams.

The results of these four groups of tests indicate that the size and weight of the material have little effect on the energy used in hemming. More data are needed before the reasons for the slight variations can be satisfactorily discussed.

In order to test the effect of speed, three tests were made on hemming blankets at 30 stitches a minute. Whereas at the rate of 18 stitches a minute the energy used was 4.3 calories per hour, at the rate of 30 stitches it rose to 7.2 calories. It is interesting to note that the ratio of increase in speed is practically identical with that in energy expenditure. One can not safely generalize from such limited data, but as far as they go these experiments indicate that the energy output is directly proportionate to the speed of work.

The results of these rapid sewing tests conform very closely with those obtained in similar tests previously made with another subject and indicate that the two individuals used the same amount of energy for the same work done at the same speed.

Experiments with machine sewing. There is undoubted practical interest in the economy of energy that comes from using foot-propelled and motor-driven machines as compared with sewing by hand, and five experiments were made to test this point, the work being done on sheets like those hemmed by hand. A well-known, high-grade make of machine was placed in the calorimeter. During three experiments it was worked by an ordinary treadle and in two others by an electric motor manufactured by the makers of the machine.

The energy required to run the machine by foot power was 33.6 calories per hour or six times as much as for hemming by hand. The energy used per meter of sewing, however, was only 0.70 calorie or considerably less than one-half that required for an equal amount of hand sewing. In the tests with the motor-driven machine the energy output for work alone was 8.3 calories per hour. This is nearly twice as much as for hand sewing, but only one-quarter as much as for the foot-driven machine. The energy required for a meter of sewing on the motor-driven machine was only 0.15 calorie, or less than one-tenth of the energy used for an equal amount of sewing by hand, and one-fifth of that on the foot-driven machine.

In other words, either type of machine sewing uses more energy per hour than hand sewing, but accomplishes much more for a given expenditure of energy. In both respects the machine driven by electricity appeared several times more economical of body energy than when driven by foot power.

SUMMARY

The respiration calorimeter was used to measure the energy expended by a woman hemming by hand on various materials and at different speeds and doing similar sewing on a machine driven by foot power and by electricity. Little variation was found in the energy required for hand hemming on fine handkerchiefs, cotton sheets, 8-ounce cotton duck, and army blankets, the energy required for the actual sewing running between 5.5 and 5.8 calories per hour except in the case of army blankets where for some unexplained reason it was only 4.3 calories.

When the speed was increased, the energy output increased proportionately. Hemming sheets on a foot-driven machine required about six times as much energy per hour as doing the same work by hand, but the energy used per meter of sewing was hardly one-half as great. When an electrically driven machine was used, the energy required per hour was not quite twice that used for hand sewing and about one-fourth that for the foot-driven machine; the energy per meter of sewing was about one-fifth of that measured on the foot-driven machine and less than one-tenth that of hand sewing. A three weeks' attack of influenza during the progress of the experiments made it possible to compare the energy output of the subject before and after the infection. For five weeks after apparently complete recovery her energy expenditure per kilogram of body weight averaged nearly 4 per cent lower than before her illness.

THE PATHWAY FOR VISCERAL AFFERENT IMPULSES WITHIN THE SPINAL CORD

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The relation of the visceral efferent nervous system to the control of the viscera has been extensively studied. Little work has been done, on the contrary, in a study of the visceral afferent fibers and their pathway through the sympathetic system, the cerebrospinal nerves and their connections in the central nervous system. The conclusions reached with reference to the afferent impulses from the viscera have been incidental to investigations of the visceral reflexes. Practically nothing can be definitely stated in regard to the course of visceral afferent impluses within the spinal cord and medulla.

The course within the spinal cord of the somatic afferent impulses which initiate vasomotor and respiratory reflexes has been investigated by Ranson, von Hess and Billingsley (1), (2). It is well known that stimulation of the sciatic nerve under appropriate conditions results in either a rise or a fall in blood pressure, varying with the strength and rate of the stimulus employed. Drops in blood pressure may be obtained from this nerve with a weak current, while a stronger results in a rise. By creating various lesions in the spinal cord of cats, the authors just mentioned showed that the somatic afferent impulses leading to pressor responses enter the spinal cord through the lateral division of the dorsal root, which consists of unmyelinated fibers, and ascend in the tract of Lissauer. The medial division of the dorsal root, which consists of myelinated fibers that enter the posterior funiculi, is not concerned in the pressor reflex. Bilateral destruction of the apex of the posterior horn and tract of Lissauer obliterated the pressor reflex from central stimulation of the sciatic nerve. The results obtained from cats with lateral hemisection of the cord and from others in which the lateral funiculi only had been divided, showed that the

pressor pathway is equally bilateral or chiefly homolateral in the apices of the posterior horn; and the depressor path is chiefly crossed and located in the lateral funiculi of the spinal cord.

It is known that many impulses from the viscera never reach consciousness but produce reflex changes in the vasomotor and respiratory systems. In this experimental work we have attempted to trace within the spinal cord, the course of the afferent impluses resulting from stimulation of the central end of the thoracic sympathetic nerve in the cat and evidenced in vasomotor and respiratory responses.

Burton-Opitz (3), working upon dogs under ether anesthesia, obtained a fall in the blood-pressure curve upon stimulation of the thoracic sympathetic nerve with a weak current. Auer and Meltzer (4), upon stimulation of the same nerve in the dog, did not obtain a pressor response in any instance. Attention may here be called to the fact that stimulation of the thoracic sympathetic nerve in the cat was found by Sollman and Pilcher (5) to yield a pressor response. It is worthy of note that stimulation of the thoracic sympathetic nerve and the vagus nerve in the cat and dog yield opposite results. Stimulation of the former yields a pressor response in the cat and a depressor response in the dog; while vagus stimulation in the cat produces a fall of blood pressure, and in the dog, a rise. The explanation of these facts has not yet been given.

TECHNIQUE. To avoid variation in results due to age, only full-grown cats were used in these experiments.

The series of investigations to determine the normal response from stimulation of the thoracic sympathetic nerve was conducted upon animals which were given urethane by an intraperitoneal injection. The dosage of the drug administered was 3 cc. per kilogram of body weight. Each 3 cc. of the solution contained 0.75 gram of the drug. Urethane was used as an anesthetic because it was found early in this work that a steady narcosis was difficult to maintain with ether and that the slightest period of deep anesthesia prevented a response upon stimulation.

After isolating the sciatic and brachial nerves on one side, the carotid artery was prepared for a blood pressure tracing. A tracheal cannula was then inserted and connected with an automatic compressed air machine which gave an interrupted supply of air at intervals corresponding to the normal respiratory rate. The pressure under which the air entered the lungs did not exceed 2 mm. Hg. In all animals both vagus nerves were divided in the neck.

Following this procedure and after mass ligation of the vessels in the chest wall, the thorax was opened upon one side parallel to the course of the seventh or eighth rib. This usually was at the level of the dome of the diaphragm. The thoracic sympathetic nerve was then isolated just before it pierced the diaphragm, ligated and divided distally. It was then carefully freed from the surrounding tissue and the rami communicantes of the 10th dorsal to the 13th dorsal segments inclusive were divided. Care was taken to avoid injury to the preparation by pinching or by exerting tension. When not being stimulated, the nerve was placed in its natural bed and was thus kept moist and warm.

A heart lever was then attached to the diaphragm and respiratory movements of this organ were recorded directly. Upward movements of the lever indicate a contraction and downward movements a relaxation of this muscle.

Stimulations were effected through an inductorium and electrode giving a tetanizing current. Experiments are now under way to determine the character of response which may be obtained with a slower rate of stimulation. The animal's legs were securely tied to the board to prevent flexion of the extremities upon the abdomen influencing the blood pressure by increasing intra-abdominal pressure. In addition to the carotid and respiratory tracings the pupils, corneal reflexes and movements of struggling were also observed.

In another series of animals, various spinal cord lesions were produced at the level of the second dorsal segment, under strict surgical asepsis. Ether anesthesia was used in these preliminary operations and the animals were allowed to recover over periods varying from 24 to 48 hours post-operative. Sterile normal salt solution was injected subcutaneously to assist in the recovery from operative shock. The lesions were made with a fine iridectomy knife and consisted of dorsal hemisections, complete lateral hemisections, sections of the lateral funiculi, leaving the posterior gray columns intact, anterior hemisections and complete transverse section of the spinal cord. All of the animals evinced signs of recovery from operative shock before stimulations were attempted. The secondary procedures varied not at all from the steps described above for the normal animals.

After death, the level of the lesion was determined accurately at autopsy and a portion of the cord including the lesion was removed and stained by the pyridine-silver method of Ranson. Serial sections were subsequently made and microscopical verification of the lesion was sought.

These experiments were conducted upon eight normal cats, five cats upon which dorsal hemisection was performed, four upon which complete lateral hemisection was performed, three cats with bilateral sections of the lateral funiculi, two cats with anterior hemisections and one cat with a complete transverse section of the spinal cord. This latter lesion was made to verify the results obtained by transecting the cords of the cats upon which normal records had been previously obtained.

RESULTS: *Normal cats.* Figure 1 is a graph obtained from stimulation of cat 15 and is typical of the results obtained from the other nor-

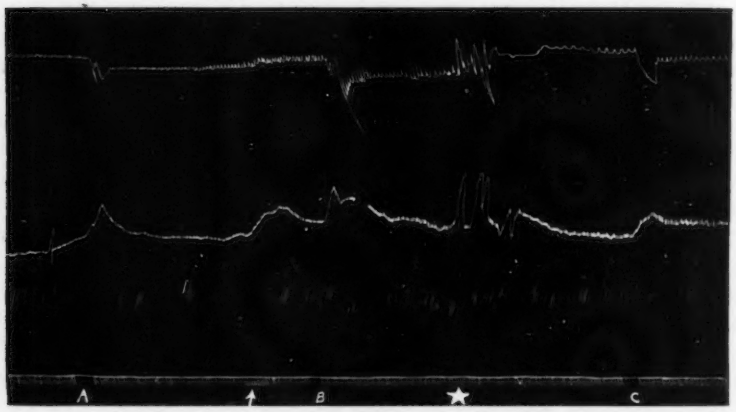

Fig. 1. Cat 15: stimulation of the thoracic sympathetic, sciatic and brachial nerves in a normal cat.

A: Thor. sym. n. s. c. 15 cm.
Arrow: Manipulation of sciatic n.
B: Sciatic n. s. c. 10 cm.
Star: Struggling.
C: Brachial n. s. c. 10 cm.

mal animals. As will be seen from the tracing, stimulation of the thoracic sympathetic nerve with a tetanizing current, the secondary coil of the inductorium being situated at 15, results in a marked pressor response. The rise and fall to normal are both fairly rapid. Coincident with the blood pressure response, the diaphragm exhibits a relaxation which is held for 1 or 2 seconds and then is followed by marked clonic contractions and relaxations. Following the stimulation, the diaphragmatic movements can be seen to be increased in rate. Stimulation of

the sciatic and brachial nerves with this type of current also produces pressor responses in the blood-pressure curve differing from the thoracic sympathetic response in that the rise is more abrupt and the fall to normal is more gradual.

Movements of the diaphragm in both cases are similar, except that the effect of stimulation is much more pronounced in the case of the

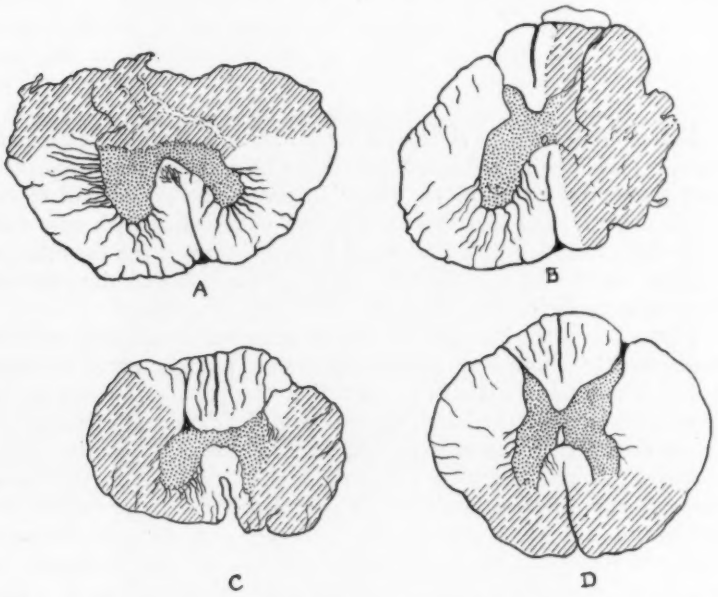

Fig. 2. Outline drawings made from pyridine-silver sections of the spinal cords of the various experimental animals, showing the types of lesions produced opposite the second dorsal segment of the cord.

A: Posterior hemisection.
B: Lateral hemisection.
C: Section of lateral funiculi.
D: Section of anterior funiculi.

somatic nerves. The results obtained upon stimulation of the sciatic and brachial nerves are in accord with those given by Ranson and von Hess (1).

During stimulation in each instance pupillary dilatation occurred. This was much more marked in the case of the sciatic, followed by the

brachial and thoracic sympathetic in the degree of dilatation. Struggling movements, which might be assumed to be evidence of pain, followed after the stimulation had ceased and were again more marked in the case of the sciatic, followed in turn by the brachial and thoracic sympathetic nerves.

At all times during the stimulation, the corneal reflexes of the animals were active and brisk. Upon no animal was stimulation of the thoracic sympathetic followed by a depressor response in the blood pressure curve. However, as stated previously, experiments are now under way using a slowly interrupted current to determine if this type of stimulation will yield a depressor reponse.

Dorsal hemisection of the spinal cord. Figure 2, *A*, is a sketch made from a pyridine-silver stain of a section of the spinal cord from cat 8 and shows the extent of the lesion involving the dorsal half of the cord. This section illustrates the lesions obtained in each case with only slight variations in extent which have no bearing upon the ultimate results. It can be seen that the posterior funiculi and the posterior gray columns have been destroyed.

Figure 3 is a tracing of the blood pressure and respiratory response occasioned by stimulation of the thoracic sympathetic nerve following dorsal hemisection. It will be seen that the pressor response in the blood pressure and the initial relaxation of the diaphragm followed by clonic contractions are exactly analogous to those obtained in normal animals. This tracing also shows the lack of response in the blood-pressure curve from stimulation of the sciatic nerve. The result obtained from the sciatic stimulation is in accord with the work of Ranson and von Hess (1), who found that dorsal hemisection destroyed the results of sciatic stimulation upon the blood-pressure curve. They found however that it did not destroy the respiratory response and while this particular tracing does not show any diaphragmatic response to to stimulation of the sciatic, we are able to verify their observations from other animals.

Lateral hemisection of the spinal cord. Figure 2, *B*, a sketch made from a pyridine-silver stain of a section of the spinal cord of cat 16, upon which a right lateral hemisection of the cord had been performed, shows the lesion to involve the right side of the cord almost completely. Only a very small area remains intact in the posterior and anterior funiculi. The lateral funiculus is entirely destroyed by the lesion. This section is representative of the type of lesions produced in this series of animals.

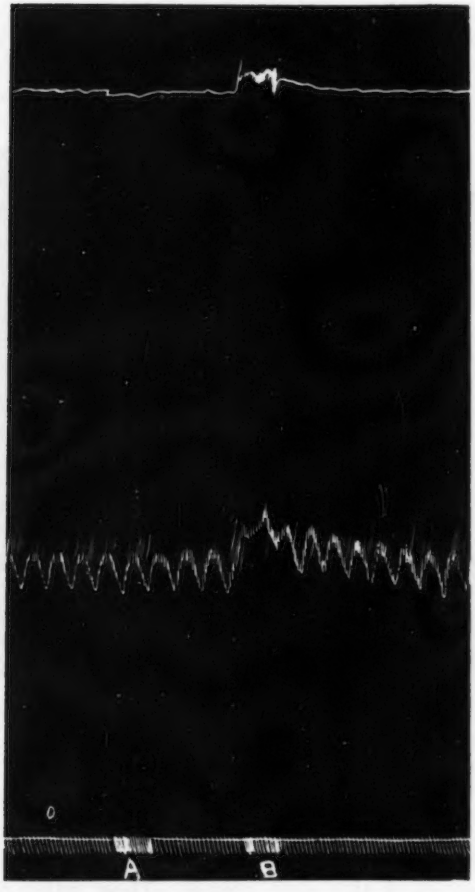

Fig. 3. Cat 12: stimulation of thoracic sympathetic and sciatic nerves 24 hours after dorsal hemisection of the cord opposite the second dorsal segment.
A: Sciatic n. s. c. 10 cm.
B: Thor. sym. n. s. c. 0 cm.

Figure 4 is a graph of the results obtained in cat 16 upon which a right lateral hemisection was performed. Stimulation of the *right* thoracic sympathetic nerve produced a rise in blood pressure accompanied by an inital relaxation of the diaphragm followed by clonic contractions. This is again in accord with the results obtained upon normal animals. Tracings 4, *B*, and 4, *C*, show the results of stimulation of the right brachial

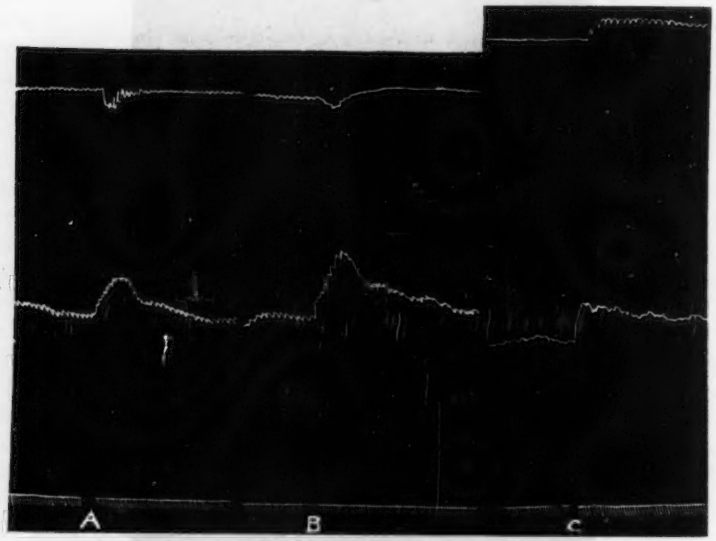

Fig. 4. Cat 16: stimulation of right thoracic sympathetic, right sciatic and right brachial nerves 24 hours after a right lateral hemisection of the spinal cord at the level of the second dorsal segment.

A: Thor. sym. s. c. 15 cm.

B: Brachial n. s. c. 7 cm.

C: Sciatic n. s. c. 6 cm.

and sciatic nerves of this same animal. These latter results again corroborate the findings obtained by Ranson and von Hess in lateral hemisection, upon stimulation of the somatic nerves, since they found that the pressor pathway from the periphery is essentially a bilateral one within the cord. Stimulation of the right thoracic sympathic nerve following a *left* lateral hemisection of the spinal cord produced exactly the same blood pressure and diaphragmatic responses.

Section of the lateral funiculi of the spinal cord. A camera lucida sketch of the lesion made at a primary operation upon cat 18 and shown in figure 2, *C*, shows a destruction of both lateral funiculi including the antero-lateral portion of the gray matter upon the left side of the cord. As will be seen, the posterior funiculi and both posterior gray columns as well as the greater part of both anterior funiculi are intact.

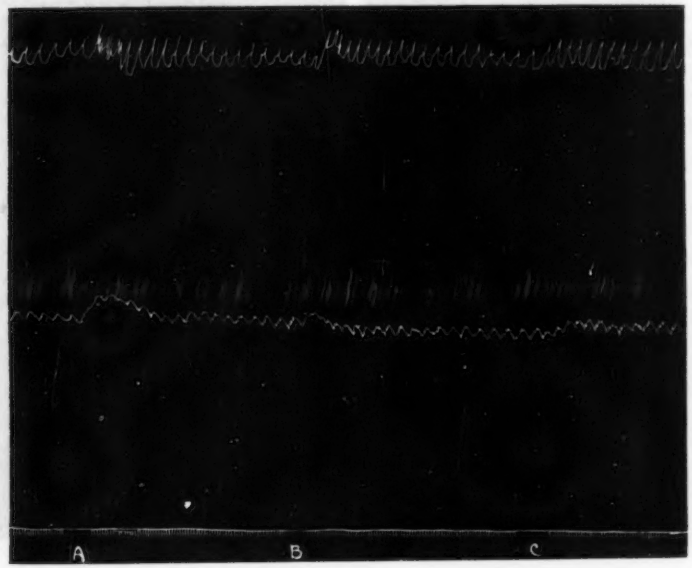

Fig. 5. Cat 18: stimu'ation of thoracic sympathetic, sciatic and brachial nerves 72 hours after a bilateral section of the lateral funiculi of the spinal cord, opposite the second dorsal segment.

A: Thor. sym. n. s. c. 3 cm.
B: Brachial n. s. c. 3 cm.
C: Sciatic n. s. c. 3 cm.

Figure 5 is a tracing made in the experiment upon this animal, 72 hours following the primary operation. We see the response to stimulation of the thoracic sympathetic nerve is still evidenced in the blood-pressure curve and in the respiratory tracing. It will be seen that there is also a pressor response upon stimulation of the brachial nerves although

the height of the curve is not such as would be expected from the intensity of the stimulus. The sciatic nerve in this animal also gave a pressor response and slow, deep respirations which are seen on the tracing. Results of this series of animals definitely show that the lateral funiculi do not contain specialized pathways for this group of afferent impluses. It will be noted in these tracings that the respiratory responses to stimulation are greatly reduced. This would tend to

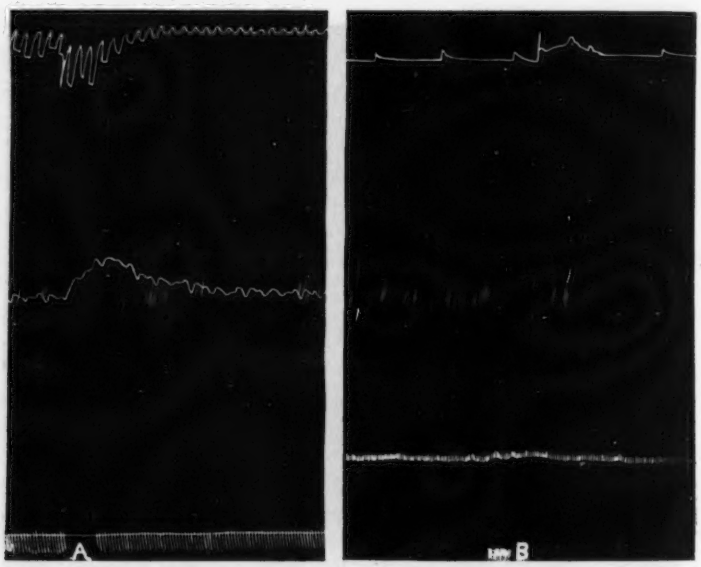

Fig. 6. *A*: Cat 22: stimulation of thoracic sympathetic nerve following section of both anterior funiculi.

B: Cat 20: stimulation of thoracic sympathetic nerve 6 hours following complete transection of the spinal cord opposite the second dorsal segment.

corroborate the view of Ranson and von Hess that the impulses leading to reflex changes in respiration pass up in the posterior one-half of the lateral funiculus.

Anterior hemisection of the spinal cord. Figure 2, *D*, is a sketch showing the lesion produced in cat 22, upon which section of both anterior funiculi was done. It will be seen that practically the entirety of both anterior funiculi has been destroyed as well as portions of the lateral tracts.

Figure 6, *A*, is a tracing obtained from stimulation of the thoracic sympathetic nerve in this animal. The customary rise in the blood-pressure curve is seen, as well as the respiratory response to stimulation. One other animal gave this same characteristic response and showed with little variation the same type of lesion.

Complete transection of the spinal cord. In addition to transection of the cord in normal animals upon which we had stimulated the thoracic sympathetic nerve just previously, we operated upon an animal and divided the spinal cord opposite the second dorsal segment 6 hours prior to stimulation. The animal had sufficiently recovered from operative shock to rule out this factor in interpreting our results. Figure 6, *B*, is a tracing obtained from this animal and the results corroborate those obtained previously. As will be seen from this tracing, there is no response in the blood pressure curve indicating a true pressor stimulus.

DISCUSSION

The results obtained in these experiments have been so uniform that no theorization is necessary. It is obvious that dorsal hemisection of the cord, which destroyed both posterior funiculi and posterior gray columns has no effect whatever upon the pressor responses obtained from the thoracic sympathetic nerve. On the contrary, this type of lesion has very definitely prevented a pressor response from stimulation of the sciatic nerve. This fact alone, therefore, rules out the posterior funiculi and any part of the posterior gray columns as the pathway for the conduction of this type of visceral afferent impulses. This is in contradistinction to the findings of Ranson and von Hess, working with the somatic nerves, since they found the pressor pathway within the cord to be situated in the apices of the posterior horns.

Neither does complete lateral hemisection cause a disappearance of the pressor response to thoracic sympathetic stimulation. This is true whether the nerve on the side of the lesion or on the opposite side is stimulated. This strongly suggests that whatever the pathway within the cord, it obviously must be one which ascends partly on the homolateral and partly on the contralateral side. Section of the lateral funiculi and including a portion of one of the anterior gray columns has resulted in a slight decrease in the height of the pressor response but the characteristic rise still remains. It must be evident therefore that the pathway is not a long crossed tract situated within the lateral funiculi; nor can the pathway be located in the anterior funiculus since

a lesion destroying these latter funiculi does not influence the blood pressure curve. The only lesion produced which has destroyed this pressor response from stimulation of the thoracic sympathetic nerve has been a complete transverse section of the spinal cord.

It is reasonable to presume, therefore, that the impulses arising from the viscera, transmitted through the thoracic sympathetic nerve and giving rise to vasomotor and respiratory responses, are conducted upwards by relays of short spinal paths with synapses in the gray matter of the spinal cord. If this be true they resemble the fibers which convey pain sensation in the cat from the periphery, since Karpus and Kreidl (6) have shown that pain is still felt in the hind limbs after section of both halves of the cat's cord at different levels. This would seem therefore to indicate that in this animal, at least, short spinal paths play a very important part in the conduction of pain. Since complete transection of the spinal cord at the level of the second thoracic segment eliminated the pressor response, it is obvious that the reflex arc is not complete within the spinal cord and that the visceral afferent impulses must reach the bulbar vasomotor center before they can become effective in producing a pressor response.

CONCLUSIONS

1. Visceral afferent impulses producing the pressor reflex are not conducted within the spinal cord along the same pathway as that involved in conducting the somatic afferent impluses giving the same reflex.

2. The visceral afferent pathway producing vasomotor and respiratory reflexes probably consists of relays of short fibers with synapses in the gray matter of the spinal cord.

3. Stimuli of sufficient intensity to produce any response from the thoracic sympathetic nerve of the cat result invariably in a rise in blood pressure. This fact is being checked up by using very slow stimulations.

4. Our experimental lesions corroborate the work of Ranson, von Hess and Billingsley, placing the afferent pressor path involved in the responses obtained from somatic nerves, in the apices of the posterior horns.

I wish to express my appreciation to Dr. S. Walter Ranson, who suggested this problem, and to Dr. Allen B. Kanavel, whose kindness has made work of this nature possible.

BIBLIOGRAPHY

(1) Ranson and von Hess: This Journal, 1915, xxxviii, 128.
(2) Ranson and Billingsley: This Journal, 1916, xl, 571.
(3) Burton-Opitz: This Journal, 1916, xli, 103.
(4) Auer and Meltzer: Zentralbl. f. Physiol., 1912, xxvi, 1316.
(5) Sollman and Pilcher: This Journal, 1912, xxx, 369.
(6) Karpus and Kreidl: Pflüger's Arch., 1914, clviii, 275.

VASOCONSTRICTION FROM WARMTH STIMULATION

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In a recent contribution from this laboratory (1) the conclusion was drawn that "gentle or limited (sensory) stimulation not arousing mental alertness typically induces vasodilatation, while reflex vasoconstriction, not dependent on psychic excitation, requires intense or widespread stimulation for its elicitation." This conclusion is in line with earlier findings, among which may be cited Martin and Lacey (2) and Martin and Mendenhall (3). This point of view, which makes the character of vasomotor responses dependent on volume of sensory stimulation, does not make allowance for certain responses which appear at first glance to be specific for the stimuli rather than related to the whole volume of stimulation. Reference is to the vasomotor effects following the application of warmth and cold to the skin. The standard picture of these effects implies, if it does not definitely claim, that stimulation of the end organs for cold elicits typically reflex vasoconstriction and stimulation of the end organs for warmth reflex vasodilatation.

If the view that a large volume of stimulation induces vasoconstriction is correct, it should be possible to elicit that reflex by applying warmth to a sufficiently large area of the skin to set up a large volume of stimuli from the receptors for warmth. That this result may actually occur was suggested to one of us (M) by the familiar observation that when one enters a warm bath, there very commonly develops immediately thereafter a pronounced contraction of the arrector pilae muscles, commonly called "goosefleshing," accompanied by a very decided feeling of chilliness, particularly in the small of the back. The goosefleshing is often very conspicuous, particularly on the arms and on areas of the legs not immersed. A series of observations on this phenomenon showed that it comes on with great promptness, developing within 5 seconds after immersion and enduring approximately 45 seconds. It was observed at bath temperatures ranging from 38°C. to 43.5°C., all these temperatures being below the threshold

for stimulation of the cold spots by warmth according to the diagram given by v. Frey (4). Although the goosefleshing and feeling of chill do not prove the onset of a concurrent vasoconstriction, they undoubtedly suggest the latter as a probable accompaniment, since, in general, conditions that induce goosefleshing or a feeling of chill are those that also favor vasoconstriction.

To put the question to a test of direct experimentation is the object of the present investigation. The marked feeling of chilliness is in the small of the back. If a drop of temperature in that region can be demonstrated immediately following entrance to a warm bath, the development of a vasoconstrictor reflex following stimulation of a large number of warmth spots seems to us demonstrated.

Technique. As a means of recording changes in back temperature we adopted the bolometer method.[1] The bolometer, which was especially constructed to fit the small of the back, consists of a double grid of bare nickel wire; the wire being approximately 0.2 mm. in diameter. The individual strands of the grid were about 1 mm. apart, very carefully placed to avoid short-circuiting. The grids were strung over a wooden block raised slightly in the middle by strips of rubber tubing to insure good contact with the surface of the back. The two grids were completely insulated one from the other and the terminals of the two led to opposite sides of an ordinary Wheatstone bridge, forming the unknown resistances thereof. The other two arms of the bridge contained respectively a resistance of 10 ohms and a slide wire resistance equipped with a micrometer set screw, enabling minute changes in resistance to be secured. A dial register was so arranged that movements of the micrometer screw were accurately indicated. The equilibrium of the Wheatstone bridge was determined by means of a moving coil D'Arsonval galvanometer of the usual laboratory type. The bolometer was calibrated by immersing it in oil at body temperature and determining the change in resistance that had to be introduced to balance the bridge for changes in temperature of 0.1°C. A calibration chart was prepared without difficulty and it was found that the bolometer would register with certainty temperature changes of $\frac{1}{1000}$°C.

We experienced some early difficulties on account of the necessity of getting our subjects into a condition of uniform skin temperature and then introducing them into the warm bath without allowing any

[1] We beg to acknowledge the courtesy of Prof. F. J. Rogers, of the department of Physics of this University, in giving us helpful suggestions in connection with our use of the bolometer.

factors other than the actual change in back temperature due to stimulation of warmth on the skin to affect the bolometer. One point in our favor was that in bringing the subject into the warm bath we were necessarily bringing the bolometer nearer to the source of heat represented by the warm water. The tendency would be, therefore, for the temperature of the bolometer to rise rather than to fall, so that any drop in temperature would be known not to be due to the simple shift of the bolometer to a different spot.

In carrying out the experiments the bolometer was secured firmly in position in the small of the subject's back by means of surgeon's tape and he was then seated on a wooden seat without legs and with a sloping back. The subject leaned against this back, thus insuring immobility to the bolometer. His seat was long enough so that as he sat upon it his legs were straight. The seat rested on a support level with the top of the tank of warm water in which the subject was to be immersed. The depth of water in the tank was such that when the subject was lowered into it, his legs were entirely below the surface, not far from half the total body area being immersed. His position in the tank was such that the part of his back to which the bolometer was applied projected beyond the edge, thus the current of warm air rising from the surface of the warm water did not strike directly upon the bolometer. The method of experimentation was as follows: with the subject in as comfortable a position as he could assume and the bolometer in place against his back the adjustable resistance on the Wheatstone bridge was shifted until the bridge was exactly balanced. An interval of a minute or two was allowed to elapse and the reading repeated to be sure that the bolometer was in equilibrium with the back temperature. The seat with the subject on it was then lifted bodily by two stout assistants and lowered carefully into the tank. With the aid of a stop watch the moment of appearing of goosefleshing was observed and simultaneously an observer at the galvanometer continually adjusted the Wheatstone bridge to compensate for alterations in back temperature as indicated by the bolometer. The significant temperature changes all occurred within one minute after immersion. The experimental results are summarized in table 1.

As the table shows, positive results were secured on six different subjects. One other, tested in precisely the same manner, gave no appreciable lowering of back temperature. Subject L, who reacted positively on three occasions, gave a negative result on a fourth. Nine of the eleven experiments we performed agree in showing that exposure

of a large area of the body to warm water is followed promptly by a drop in temperature in the skin of the small of the back. The average drop was slightly less than 0.2°C. The latency averaged 7 seconds. In three of the experiments the bolometer registered a slight initial rise in temperature, preceding the usual drop.

TABLE 1

Table showing the degree of lowering of back temperature following immersion of the nether half of the body in warm water

DATE, 1921	ROOM TEMPERATURE	BATH TEMPERATURE	LATENCY OF GOOSE-FLESHING	LATENCY OF TEMPERATURE DROP	TEMPERATURE DROP	CHILL FELT	SUBJECT
	°C.	°C.	*seconds*	*seconds*	°C.		
May 13	23.4	41.7	8.4	10.2	0.188	Uncertain	P.
May 17	21.0	42.0	2.3	5.5	0.115	Uncertain	L.
May 17	21.3	42.0	2.1	3.1	0.212	Yes	C.
May 19	20.5	42.0	7.6	8.0	0.258	Yes	L.
May 19	21.0	41.0	5.6	7.0	0.199	Yes	D.
May 20	22.0	41.0	5.3	7.4	0.104	Yes	L.
May 24	22.0	41.5	?	18.0	0.122	Yes	G.
May 25	22.0	41.5	1.6	3.0	0.190	No	S.
May 25	22.0	42.0	2.2	4.0	0.251	Yes	D.

DISCUSSION

Correct interpretation of the functioning of the nervous system hinges in part on the question of whether such "centers" as the vasoconstrictor or vasodilator are tied up in an especially intimate manner with particular groups of sense organs, so that stimulation of the sense organs can be depended on to cause reflex discharge of the centers with which they have this intimate connection. According to the view in course of development in the group of papers of which this is one, such special relationships are less numerous than our standard pictures of nervous functioning would seem to imply. Since one of the most frequently cited illustrations of this type of relationship is the vasodilatation following stimulation of warmth, as contrasted with the vasoconstriction accompanying cold, it seemed that analysis of these reflexes should be particularly instructive.

In the experiments cited herewith we have what seems to us convincing evidence, reinforcing that previously offered elsewhere (1), to the effect that volume of stimulation, rather than origination in particular

sense organs, is what determines which of the two centers, the vasodilator or vasoconstrictor, is to be excited to discharge. To the possible objection that our observed temperature drops were too slight to be outside the limits of error we would reply that the concomitant phenomena, goosefleshing and feeling of chill, phenomena that can be observed by anyone at any time, are in themselves evidence of nervous discharges in the same category with vasoconstriction, so that any carefully performed experiments actually demonstrating a lowering of temperature have more force than would be the case otherwise.

Moreover, the view of Martin and Mendenhall (3) should be recalled, according to which vasoconstriction, as it develops, overpowers concurrent vasodilatation; a view which they support by showing that the latency of the latter reaction is briefer than of the former, so that with fortunately selected stimulation a preliminary dilatation followed immediately by constriction can be demonstrated. If in the experiments here reported the reflex response to the stimulation of warmth had actually been vasodilatation instead of vasoconstriction there should have been from the beginning a rise in back temperature, in no case followed by a fall during the time of immersion.

The fact that the lowering of back temperature was only momentary presents no difficulties when we remember that the temperature of the blood must have commenced to rise very shortly after immersion in the warm water. The effect of a rise in body temperature in inducing cutaneous vasodilatation is too familiar to call for comment. That it, rather than a vasomotor reflex, is the usual cause of cutaneous flushing we think our observations indicate.

Although we have no observations on the effect on body temperature of warming small areas we would call attention to the fact, easily verifiable, that under normal conditions neither goosefleshing nor feeling of chill result therefrom. One of us (M) has made on himself the observation, however, that when suffering from a condition predisposing to "chills" slight sensory stimulation often suffices to arouse marked goosefleshing, accompanied by feeling of chill. This is probably the common experience, although we have not seen it commented on in the literature. It suggests an increased susceptibility of the vasoconstrictor mechanism, causing it to discharge under less stimulation than is normally required.

SUMMARY

In nine out of eleven experiments immersion of the lower part of the human body in water at about 42°C. was followed in 7 seconds, on the average, by a drop in temperature of the skin of the small of the back averaging slightly less than 0.2°C. Accompanying this drop in temperature were well-marked "goosefleshing" and sensations of chilliness. We interpret these findings as evidence that a large volume of nervous discharge, due to stimulation of a great many receptors for warmth, elicits, typically, reflex vasoconstriction. This interpretation is opposed to the view that vasodilatation is the specific reflex response to stimulation of the end organs for warmth.

BIBLIOGRAPHY

(1) MARTIN, FRANKLIN AND HIELD: This Journal, 1920, liii, 421.
(2) MARTIN AND LACEY: This Journal, 1914, xxxiii, 212.
(3) MARTIN AND MENDENHALL: This Journal, 1915, xxxviii, 98.
(4) v. FREY: Ergebn. d. Physiol., 1910, ix, 360.

THE APPLICATION OF THE "ALL OR NOTHING" PRINCIPLE OF NERVOUS CONDUCTION TO THE INTERPRETATION OF VASOMOTOR REFLEXES

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Serious students of the functioning of the nervous system are agreed that the "all or nothing" conception of nervous conduction, as developed by Lucas (1) and Adrian (2), must be, if not dogmatically accepted as a tenet of physiological thought, at least taken into account in any attempt to picture the machinery of nervous activity. So far as I am aware, no systematic attempt to analyze vasomotor reflexes in terms of this principle has yet been made, and in the hope that such an analysis may prove instructive this paper is submitted.

Experimental study of vasomotor reflexes has been carried on, for the most part, with the aid of responses elicited artificially through the stimulation of naked nerve trunks with induction shocks. Unphysiological though this procedure may appear to be, in that it subjects the experimental animal to conditions which it could not possibly encounter normally, the method has the great advantage over receptor stimulation that it enables the observer to determine much more reliably what is actually happening in the stimulated area. Numerous investigators have studied the response of the nerve trunk to electrical stimulation (for references to the literature see Lucas, *The conduction of the nervous impulse*, London, 1917), while our knowledge of the activities set in motion in the nerve trunk as the result of receptor stimulation is extremely meager.

According to the view of Lucas and Adrian, which for the purposes of this discussion is accepted as completely demonstrated, the effect of an adequate electrical stimulus upon a single nerve fiber is to set up in it a nervous impulse which is propagated along the fiber from the point of excitation with an intensity that is determined solely by the condition of the fiber at the moment, and without reference to the intensity of the exciting stimulus. This conception is the essence of

the "all or nothing" principle. When the stimulus is applied to a nerve trunk, containing, as it may, hundreds of fibers, the stimulating current divides itself among the numerous conducting pathways presented to it according to the law of divided circuits, and it will follow almost inevitably that some nerve fibers in the trunk will receive more of the current than others. Thus it may happen that some of the fibers will be adequately stimulated, and in these full-size nerve impulses will be generated; other fibers may not receive enough of the current to serve as effective stimulation, and in these no impulses will be aroused. Evidently, the stronger the induction shock the larger the proportion of the fibers to be adequately stimulated, and with sufficient current in the shock all of them should be made active. That this situation actually obtains in nerve trunks has been demonstrated by Forbes and Gregg (3).

The criterion by which Forbes and Gregg concluded that all the fibers of the nerve trunk were active was the attainment in the nerve trunk of a maximal value of the action current. This maximum was reached in their experiments with a strength of shock amounting to about 50 Z units (Martin scale (4)). It must be confessed that the limit of reflex response is by no means reached with this strength of stimulation, so that if Forbes and Gregg are correct in their view that all the fibers are excited at the strength of stimulation at which the maximum action current is reached, acceptance of the "all or none" principle virtually compels the conclusion to which they come (loc. cit., p. 220), that stronger shocks may set up *successions* of impulses in nerve fibers, which may have central effects very different from those exerted by single impulses.

Do spinal nerves contain specific pressor and depressor fibers? Latschenberger and Deahna (5) appear to have been the first to postulate specific pressor and depressor[1] fibers in nerve trunks in general. Their view was later adopted by Hunt (6), who reiterates his original position in a recent contribution (7). On the basis of observations on the effect of stimulations near the threshold, Martin and Lacey (8) came to the conclusion that the strength of the stimulation is of more significance than the excitation of any particular kind of fiber, and although they

[1] These are probably excito-dilator fibers according to Hunt's terminology, in that they act to excite the dilator center, rather than to inhibit the constrictor. The word depressor is used throughout this paper as synonymous with excito-dilator, to harmonize with the terminology used by Ranson and his co-workers (9), (10).

do not directly repudiate the idea of specific pressor and depressor fibers, the tenor of their paper is against the conception. Ranson and Billingsley (10), in a research in which they establish the important fact that pressor and depressor excitations travel different paths up the spinal cord, express definitely the opinion that the weight of evidence is against the existence of specific fibers.

The contention of Martin and Lacey, which is supported by Ranson and Billingsley, is that as a general rule weak sensory stimuli are depressor, while strong stimuli are pressor. Martin and his associates used in all their investigations rather slow rates of stimulation. This was done because the primary point in the mind of these workers was the use of shocks of known intensity, and, as Erlanger and Garrey (11) have pointed out, in some induction coils overlapping, affecting the intensity, may occur if the shocks are repeated as often as 15 per second. Furthermore, to avoid polarization, the make shocks were not short-circuited, their physiological intensity being known to be only about one-eighth that of the breaks, but it was necessary to allow time between shocks for them to decay completely, so that the intensity of the succeeding break shocks might not be affected.

An adequate explanation of the differences in experimental results of Martin and Lacey on the one hand, and those investigators, Hunt among them, who have difficulty in obtaining lowering of blood pressure with weak stimulation, was afforded by Gruber (12), who demonstrated that rapid rates of stimulation (20 per second) tend to cause pressor responses whether the individual stimuli are weak or strong, while if sufficiently slow rates are used (one a second, or one in 2 seconds) very strong shocks may bring about reflex lowering of pressure. This observation of Gruber, interpreted in accordance with the "all or nothing" principle, seems to me, contrary to the idea of Hunt (loc. cit. (7), p. 246), sufficient in itself to overturn the conception of specific pressor and depressor fibers in spinal nerves, while harmonizing very well, as will be shown, with the contention of Martin and Lacey and Ranson and Billinglsey that the significant thing is the strength of stimulation. Gruber showed that a stimulus of approximately 11 Z units caused only lowering of pressure when the rate was 4 per second, while the same stimulus usually resulted in reflex rise of pressure when the rate was increased to 20 per second. According to the doctrine of specific pressor and depressor fibers different fibers were stimulated by the rapidly succeeding shocks than by the slower. There seems no way, in terms of the "all or nothing" principle, by which this shift

of effect from one set of fibers to another could come to pass as the result of an increase in rate, except through some process of summation of excitation. But Lucas (13) has studied this latter phenomenon carefully and finds that the duration of summation of excitation in nerve trunks does not exceed 0.005 second at ordinary temperatures, an interval much too brief to be of significance here. Furthermore, the observation of Gruber that very strong shocks sent in at long intervals result in lowering of pressure is inconsistent with the idea of specific fibers, since shocks that are able to stimulate particular pressor fibers should not lose that power merely by being sent in less frequently. I see no way in which these observations can be reconciled with a theory of specific pressor and depressor fibers if the "all or nothing" principle is accepted.

Certain observations bearing on this same problem from the standpoint of receptor stimulation have recently been made in this laboratory (14), (15). Martin, Franklin and Hield showed that in rabbits a fairly clear relationship can be shown to exist between the intensity or extent of the receptor stimulation and the character of the vasomotor response, feeble or restricted stimulation causing typically vasodilatation, while intense or widespread stimulation tends to evoke vasoconstriction. Martin and Jacoby, in a paper accompanying this, present evidence to the effect that a type of sensory stimulation generally regarded as almost specifically depressor in action, namely, warmth on the skin, induces vasoconstriction when applied over a sufficient area. If there are any specific depressor fibers those mediating sensations of warmth would seem most certain of falling within the category, yet as these experiments show, if a sufficiently widespread area is included in the stimulation. the response is that characteristic of large volume of stimulation, namely, vasoconstriction. The results both of nerve-trunk excitation and of receptor stimulation are thus seen to oppose the conception of specific pressor and depressor fibers. The alternative view, which seems to me wholly logical, is that all sensory fibers can take part in mediating vasomotor reflexes of either sort, the character of the response being determined by the nature of the impulse-stream. I propose in the next section to analyze the observations of Martin and Lacey, Ranson and Billingsley, and Gruber, cited above, together with such other significant evidence as is at hand, in the hope of being able to show what are probably the cardinal features of impulse-streams that evoke vasodilatation on the one hand and vasoconstriction on the other.

The analysis of excitations in terms of impulse streams. In introducing this section it is desirable to recall the view of the effect of stimulation upon nerve-trunks necessitated by the "all or nothing" principle of nervous conduction. Weak stimulation induces full-sized impulses in a few of the fibers, only those being involved that are most favorably situated with respect to the source of the stimulus. With stronger stimulation more and more fibers are included, until ultimately a strength of excitation will be reached which arouses impulses in all the fibers. Still stronger stimuli, according to the view of Forbes and Gregg (loc. cit.), arouse successions of impulses in some or all of the fibers, instead of single impulses as do less intense disturbances. For the sake of completeness the statement should be added that in cases where these successions of impulses are aroused the individual impulses, following the first, might fall in the relative refractory phases of those immediately preceding them, in which case they would have less magnitude than do single impulses in the normal nerve fiber.

The outstanding fact about depressor (excito-dilator) excitation, as emphasized by Gruber (loc. cit.), is that it depends upon a slow stimulation rate. Stimuli following one another at so long an interval as two seconds were effective in evoking vasodilatation in his experiments and the same response was obtained in more than 80 per cent of his reported observations at rates of 4 and 8 a second with strengths of stimulation ranking as weak or moderate. This finding is in entire agreement with that previously reported by Martin and Lacey (loc. cit.). A significant point made by Gruber, which Martin and Lacey failed to note, is that of the depressor effect of very strong stimuli when sent in at very slow rates, an effect contrasting sharply with the pressor influence of strong stimuli when sent in at moderate or rapid rates. Strong stimuli, according to the "all or nothing" principle, are those that arouse impulses in all the fibers of the nerve-trunk, and perhaps successions of impulses in some or all. Applying this definition to Gruber's experiment we have the statement that the arousing once every second or once every 2 seconds of impulses in all the sensory fibers of the nerve trunk, with perhaps *impulse-streams* in some or all as well, has the effect of evoking reflex vasodilatation. His other finding, in corroboration of Martin and Lacey, of the depressor effect of moderate stimuli at rates of 4 to 8 a second, may be stated in similar terms as follows: that the arousing of impulses in part of the fibers of the nerve-trunk 4 to 8 times a second has also the effect of eliciting reflex vasodilatation. The depressor influence is thus seen to depend on the passage of impulses

along all the sensory fibers at long intervals, or along part of them with somewhat greater frequency, but the impulse-stream as a whole, when taken for the entire period of stimulation, usually 30 seconds to one minute, must be considered moderate.

In a recent paper Hunt (loc. cit. (7) p. 244) brings up again his experiments on cooled nerves, taking the position that Gruber's observations on rate of stimulation tend to support his former views in favor of specific depressor fibers. According to the "all or nothing" principle the effect of cooling on a nerve-trunk can be only to abolish conductivity in part of the fibers without impairing it effectively in any of the others; an essential of the principle being that impulses which are able to traverse a region of impaired conductivity regain full strength on reëntering a normal area. So far as cooling has any effect at all, therefore, it must consist in extinguishment of the impulses in part of the fibers. A strong stimulus applied to such a cooled nerve arouses full sized impulses in all the fibers, but most of them are extinguished in the cool region, leaving to pass on to the spinal cord full-sized impulses in only a few fibers. This makes the situation precisely similar to that which prevails when weak shocks are used, namely, the passage of full-sized impulses over a few fibers. So that the statement of Martin and Lacey (8, p. 222), that "the effect on the nerve trunk of Hunt's procedure (cooling) was merely impairment of conductivity," is strictly correct, and the depressor effect of such stimulation is to be explained in the terms used in this discussion, as due to the fact that with so few fibers functioning the impulse volume reaching the cord in a unit of time is never more than moderate, and the central effect must always be that due to a moderate volume, namely vasodilatation. The depressor effect of stimulating a regenerating nerve is to be explained in exactly the same manner, namely, the presence in the regenerating trunk of only a few functioning fibers.

Reflex vasoconstriction, on the other hand, results from conditions which lead to the passage of a large volume of impulses along the nerve trunk during the period of stimulation. One way of realizing this is by the rapid repetition (20 a second) of weak stimuli, causing impulses to follow one another in quick succession along a few fibers. Another way is by strong stimulation at moderate intervals, 4 to 8 a second, causing impulses to pass along all the fibers of the nerve-trunk with these frequencies. It is noteworthy that according to the finding of Martin and Lacey (loc. cit., p. 222) confirmed by Gruber and Kretschmer (23) adequate pressor stimuli at these slow rates must be of a strength

(250 or more Z units) sufficient to arouse *successions of impulses* (see above, p. 401), so that the actual volume of the impulse stream within the nerve trunk is greater than that represented by a single nerve impulse for each fiber at each stimulation. Although the point has not, to my knowledge, been systematically investigated, it is reasonable to anticipate that at intermediate rates, 10 to 16 a second, perhaps, pressor reflexes might be elicitable with strengths of stimulation just sufficient to arouse single impulses within each fiber of the trunk at each stimulus, without generating successions of impulses within any of them.

The feature of cardinal importance about the above analysis of the difference between depressor and pressor excitations is that it makes the effect produced within the central nervous system dependent upon the activity of the nerve trunk as a whole, without reference to which particular sensory nerves within it are involved. In very general terms, the statement would be to the effect that the determining factor is the product of the number of active nerve fibers by the stimulation rate, or in other words, that the number of nervous impulses to arrive within the central nervous system in a given time from a given area of the periphery establishes the vasomotor response. This conception differs sharply from the familiar picture of closely linked and clearly differentiated neuron chains by which Sherrington (16) and others have postulated the machinery of skeletal muscle reflexes. Although I do not know that there has been any tendency to carry over the ideas derived from the study of skeletal muscle reflexes as a means of explaining the reflexes of the autonomic system, it seems to me worth while to emphasize the fundamental feature of difference between the two kinds of reflex; the one a definitely localized and elaborately coördinated response to a particular source and intensity of stimulation; the other an adjustment to a change in bodily state, such as manifests itself in a generalized stream of nervous influences and in which, therefore, the response may properly be to total volume of stimulation rather than to excitation localized in one or a few receptors.

The central mechanism of vasomotor response. In the section above I have dwelt upon the view that the character of the vasomotor reflex response is determined by the impulse-volume arriving within the central nervous system in a given time from the seat of excitation. If the volume is moderate or small, vasodilatation is evoked; if large, vasoconstriction. It is now in order to turn to the central nervous system itself to see how, conceivably, this may be brought about.

Since Ranson and his co-workers have shown (9), (10) that the afferent tracts within the spinal cord are different for pressor and depressor (excito-dilator) excitations, it is evident that we must look to the origins of the intra-spinal afferent tracts for the means by which impulse-streams of small volume are directed along the depressor pathway while those of large volume find their way along the quite different pressor tract.

The former of these presents no greater difficulty than confronts us in any attempt to picture, in terms of the "all or nothing" principle, how particular tracts within the central nervous system come to be the "preferred paths" for any reflex actions. If we suppose the "depressor tract" of Ranson to be freely open to impulse-streams arriving by way of the spinal nerves we can see how such streams can excite the vasodilator center. It is true that to account for the remarkable fact brought out by Gruber (loc. cit.), that stimulation separated by as long intervals as 2 seconds may still be effective in evoking vasodilatation, exceptionally prolonged summation-properties must be attributed to some portion of the mechanism (see Sherrington (16), p. 37), but otherwise we need not postulate any special characteristics for this tract.

When we turn our attention to the "pressor" pathway, on the other hand, we find ourselves under the necessity of trying to form some idea of how a tract that is not excited by a small total volume of nerve impulses becomes active under the influence of a large volume of them. Furthermore, the tract must be so organized that whether the large volume consists of rapid successions of impulses arriving over a few sensory neurons, or more slowly succeeding impulses arriving over many neurons, the effect upon it is the same. To my mind this suggests that the tract originates in a grouping, or interdependence, of synapses of such a sort that none of the synapses can be crossed by nervous impulses except as the conditions are fulfilled whereby the entire group becomes penetrable.

In certain respects the statement just made is applicable to the accepted conception of a "nerve-center," such as the vasoconstrictor center itself. The conception of spinal cord vasomotor centers is a familiar one in physiology, although not all authors accept it unreservedly (Burton-Opitz, (18)). These are ordinarily pictured as efferent to the chief vasoconstrictor center in the bulb, and as coming into function after injury to or destruction of the bulbar center. It is conceivable that the afferent "centers" which I am postulating here as the most satisfactory means of picturing the mode by which junction

is established between peripheral sensory nerve trunks and the spinal cord pressor tract, may relate themselves in some manner to the efferent centers in connection with the establishment of independent function in the latter as the result of injury to the bulb.

Granting the existence of these synapses groupings or afferent "centers" as the connecting links between the peripheral sensory nerve trunks and the intra-spinal pressor tracts, we are provided with means whereby nerve trunks acting as units can excite spinal cord tracts, which also, presumably, act as units, thus enabling equivalent effects to be produced by equivalent volumes of nervous impulses, whether these arrive over few or many peripheral neurons.

We have next to consider the significance of the fact that large volumes of nervous impulses are necessary for the excitation of pressor reflexes. This might be accounted for on the simple view that the synapse-groups or "centers" which initiate the spinal pressor paths are so resistant that only impulse-streams of large volume can cross them. A different idea is expressed by Ranson and Billingsley (loc. cit., p. 24) and by Ranson (24) who emphasize the fact that the pressor pathway, as worked out by them, is a "short tract," consisting of a succession of short neurons, and including, therefore, numerous synapses, and who advance the suggestion that the high threshold of pressor reflexes may be due to the great resistance of these numerous synapses, a resistance requiring correspondingly powerful stimulation for its overcoming. If the implication is involved that the synaptic resistance is cumulative, becoming greater as successive synapses are reached, the idea is out of harmony with the "all or nothing" principle, for according to this principle resistance to the movement of nervous impulses cannot be cumulative. Nervous impulses once initiated within any pathway proceed with undiminished intensity until a "region of decrement" (18) is reached, where the intensity falls off progressively; but if the impulses succeed in passing the region of decrement without extinction they regain full intensity upon emerging into a normal region. Synapses are regions of decrement, according to Lucas and Adrian, and it is reasonable to suppose that the presence of numerous such along a nervous pathway increases the likelihood that impulses traveling along the pathway may suffer extinction before reaching its end. Successions of impulses have been shown by Sherrington (loc. cit., p. 37) to be often much more potent in passing across synapses than single impulses. Thus it may readily be the case that a large volume of impulses can traverse a pathway including many synapses more successfully than a

small volume, but the difference is not properly attributable to the cumulative resistance of the tract as a whole.

One other point stressed by Ranson and Billingsley, and earlier by Martin and Mendenhall (19) is of the longer latency of pressor than of depressor reflexes. This the former authors, justly, it seems to me, attribute to the greater number of synapses in the pressor tract.

A feature of the interpretation of vasomotor reflexes in which I am in harmony with Ranson but in opposition to Bayliss (20) is the application to these reflexes of the principle of reciprocal innervation. In an earlier paper with Mendenhall (19) I raised the point whether the evidence does not suggest the overwhelming of vasodilatation by the concurrent constriction rather than an inhibition of the former in connection with the development of the latter. The evidence advanced by Ranson and Billingsley (loc. cit., p. 31) to the effect that after section of the pressor spinal cord path strong stimulation gives very pronounced vasodilatation seems to me wellnigh conclusive in support of the view of Ranson and of myself, for here we have an experiment similar to those used by Sherrington (p. 88) in his most convincing demonstrations of reciprocal innervation in skeletal muscle reflexes, but with precisely contrary results. The essence of Sherrington's experiments is the demonstration of reflex relaxation of the antagonistic extensor muscle after the flexor has been severed from its connection with the joint, the exciting stimulus being that favorable to contraction of the flexor. In the experiment of Ranson and Billingsley the exciting stimulus is favorable to vasoconstriction, and if the principle of reciprocal innervation applies to the mechanism, the response, after section of the pressor afferent tract, should be a lessened activity of the dilator center. What actually occurs is precisely the contrary, namely, heightened activity of the dilator center.

The gradation of vasomotor reflexes. Vasomotor reflexes share with other reflexes the property of showing gradations in degree in harmony with variations in intensity of the exciting stimulation. W. T. Porter and his associates (21) have studied this gradation with care in the case of vasomotor reflexes, and have shown that the increase of response with increased stimulation is so adjusted as to permit the construction of a curve for expressing the relationship between them. Forbes and Gregg (loc. cit., p. 234) discuss a similar gradation of skeletal muscle reflexes. These latter investigators offer a tentative suggestion to account for the gradation which occurs when the strength of stimulation is greater than that which just suffices to excite all the fibers. Their

idea, to which reference has already been made, is that strong shocks may arouse successions of impulses within individual nerve fibers. The impulse-stream set up in a nerve-trunk under strong stimulation might be pictured according to this idea as made up of successions of impulses in those fibers so situated as to receive large fractions of the total current in the shock, and single impulses in such as receive smaller fractions. It is obvious that every alteration in strength of exciting current might vary the proportion of fibers which have successions of impulses set up within them, and so the total number of impulses to be set traveling along the nerve trunk. Thus the widest possible gradation in the number of impulses arriving in the spinal cord in a unit of time becomes possible, particularly when to alterations in strength of individual stimuli are added alterations in rate of stimulation.

Attempts to explain in detail gradations within the central nervous system are too speculative in the present state of our knowledge to be very profitable, so that only outline suggestions will be offered here, and those rather with the idea of marshaling in order the facts to be taken into account than in the hope of presenting a hypothesis of central nervous action. Any picture of the mechanism by which graded vasomotor responses are secured must keep in the foreground the difference in qualitative effect between small volume and large volume of stimulation, the former depressor and the latter pressor; as well as the twofold pathway up the cord through which the two types of effect are brought about. A proper question at the outset is whether both kinds of vasomotor response are graded. The observations of Porter referred to above deal only with pressor reflexes. Martin and Stiles (22) have investigated depressor (excito-dilator) reflexes with the question of gradation in mind, and conclude that whatever gradation exists is limited to a small range. Their work was done chiefly upon the vago-depressor trunk in the cat, which ordinarily induces only lowering of blood pressure, no matter how strongly it is stimulated, so that their observations were not complicated by the possibility of concurrent pressor excitation, which by opposing the depressor influence might prevent gradation of this influence from showing itself, even when present. They point out that even in spinal nerves, which give pressor effects when strongly stimulated, the whole gamut of depressor reaction may be run, without any indication of opposition by concurrent pressor excitation, merely by keeping the strength of stimulation below the threshold for the latter, a threshold which is rather high at the slow rates of stimulation used by them, and that in such experiments little evidence of gra-

dation appears. Hunt (7, p. 245) reports an experiment with a cooled nerve in which the depression obtained by slow stimulation was not affected by cooling, while the pressor effect of a "tetanizing" current was converted by cooling to a depression equal to that obtained by means of the slow rate of stimulation. He comments upon the fact that the maximum effect was obtained from the slow rate, and was the same before as after cooling. Since, on the "all or nothing" principle, the effect of cooling must have been to cut down the volume of impulses traversing the nerve-trunk, this observation of Hunt suggests the absence of gradation in the depressor response.

Vasoconstrictor reflexes, on the other hand, as stated a moment ago, show very definite grading, a marked feature of which is its close adjustment to the strength of the exciting stimulus. This close adjustment would offer great difficulty of interpretation in terms of the "all or nothing" principle of nervous conduction, if the attempt were made to base it on the activity of specific pressor sensory fibers. By making use of the conception of total impulse-volume, however, no particular difficulty is encountered, for there is an intimate relation between strength of stimulus and resulting impulse-volume, as already emphasized (p. 410), and if we admit that different *impulse-volumes* may arouse the vasoconstrictor center to different degrees of activity, the close adjustment of the response to the *strength* of the *exciting stimulation* may be granted as well.

SUMMARY

1. The application of the "all-or-nothing" principle of nervous conduction to the question of whether there are specific pressor and depressor fibers in spinal nerve trunks leads to the view that such specific fibers cannot be postulated in the present state of our knowledge.

2. From consideration of the reflex vasomotor responses to excitation of nerve trunks with various strengths and rates of stimulation the conclusion is drawn that the significant factor is the total impulse-stream generated within the afferent portion of the nerve trunk in a unit of time. Impulse-streams of small or moderate total volume induce reflex vasodilatation; impulse-streams of large total volume induce reflex vasoconstriction.

3. In view of the finding of Ranson and his co-workers that the afferent intraspinal tracts are different for depressor (excito-dilator) and pressor excitations, it is necessary to postulate such differences between the tracts that moderate impulse-streams pass successfully

along the depressor tract to the vasodilator center, while only impulse-streams of large volume are conducted along the pressor tract to the vasoconstrictor center. The suggestion is offered that such special properties as are necessary to account for the differences in conductivity of the two pathways, may all be assigned to the pressor tract. The idea of Ranson and Billingsley that the peculiar properties of the pressor tract are due to its construction as a "short tract" is examined and criticized in the light of the "all-or-nothing" principle.

4. The question of whether the principle of reciprocal innervation applies to vasomotor reflexes is discussed and a negative view expressed.

5. The gradation of vasoconstrictor reflexes in accordance with the strength of the exciting stimulus is found to be explicable in terms of impulse-stream volume. Vasodilator reflexes show little or no evidence of gradation.

BIBLIOGRAPHY

(1) Lucas: The conduction of the nervous impulse, London, 1917.
(2) Adrian: Journ. Physiol., 1914, xlvii, 460.
(3) Forbes and Gregg: This Journal, 1915, xxxix, 172.
(4) Martin: The measurement of induction shocks, New York, 1912,
(5) Latschenberger and Deahna: Arch. f. d. gesammt. Physiol., 1876, xii, 157.
(6) Hunt: Journ. Physiol., 1895, xviii, 381.
(7) Hunt: This Journal, 1918, xlv, 231.
(8) Martin and Lacey: This Journal, 1914, xxxiii, 212.
(9) Ranson and von Hess: This Journal, 1915, xxxviii, 128.
(10) Ranson and Billingsley: This Journal, 1916, xlii, 16.
(11) Erlanger and Garrey: This Journal, 1914, xxv, 377.
(12) Gruber: This Journal, 1917, xlii, 214.
(13) Lucas: Journ. Physiol., 1910, xxxix, 466.
(14) Martin, Franklin and Hield: This Journal, 1920, liii, 421.
(15) Martin and Jacoby: This Journal, 1922, lix, 394.
(16) Sherrington: The integrative action of the nervous system, New Haven, 1906.
(17) Burton-Opitz: Text book of physiology, Philadelphia, 1920, 413.
(18) Adrian: Journ. Physiol., 1912, xlv, 389.
(19) Martin and Mendenhall: This Journal, 1915, xxxviii, 98.
(20) Bayliss: Proc. Royal Soc. London, 1908, lxxx, B, 339.
(21) Porter: This Journal, 1910, xxvii, 276.
(22) Martin and Stiles: This Journal, 1914, xxxiv, 106.
(23) Gruber and Kretschmer: This Journal, 1918, xlvi, 222.
(24) Ranson: Physiological Reviews, 1921, i, 498.

THE HYDROLYSIS OF SUCROSE IN THE HUMAN STOMACH

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The question of the extent of the hydrolysis of cane sugar in the stomach under normal conditions is still an open one. While it has been shown repeatedly and is generally conceded that the acidity of the gastric juice may be sufficient to effect a considerable degree of hydrolysis over prolonged periods of time, the actual extent to which this acid hydrolysis may proceed under normal conditions of diet in man has not been the subject of careful experimental study.

Rubner (1) and Bernard (2) stated that gastric juice might cause inversion of cane sugar, while Hoppe (3), Külz (4) and Worm-Müller (5) could observe no hydrolysis of sucrose by gastric juice in vitro. In 1887 Seegen (6), as a result of the examination of the digestive tracts of dogs, to whom 100 grams of cane sugar had been fed daily for 7 to 8 days previous to death, concluded that sucrose was completely inverted in the stomach. Lusk in Voit's laboratory (7) administered 30 grams of sucrose to a rabbit and examined the intestinal tract 6½ hours later. Ninety per cent of the carbohydrate present in the stomach was invert sugar and only 10 per cent sucrose. Traces of sucrose were found in the duodenum. Less than 20 per cent of the total carbohydrate administered was recovered from the tract. Ferris and Lusk (8) studied the hydrolysis of sucrose by hydrochloric acid in concentrations corresponding to those of normal gastric juice, and decided that the acidity of the stomach was sufficiently great to account for the hydrolysis observed in previous experiments in vivo with rabbits.

Those who have held that the hydrochloric acid of the gastric juice is the active agent in the hydrolysis of sucrose have based their belief on experiments carried out in vitro in solutions whose acidity was comparable to that of gastric juice. Two other factors however must be taken into consideration when the extent of the hydrolysis in vivo is under discussion, the rate of discharge from the stomach which is a factor limiting the time during which the acid is active upon the sucrose,

and the intestinal regurgitation. Regurgitation from the intestine is now known to be a normal phenomenon (9) and the possibility that the sucrase of the intestinal juice may be active for a short time in the stomach and, in combination with the acid, effect a greater degree of hydrolysis than is usually assumed, must be considered. It has therefore seemed of interest to carry out a series of direct experiments on the inversion of sucrose in the human stomach. The use of the Rehfuss tube makes such a study possible not only because the examinations are made under normal conditions of digestion, but also because it is possible to follow the course of digestion by making use of the fractional method of analysis.

Experimental. The methods used were in general similar to those employed in a study (10) of the behavior of inulin in the digestive tract previously carried out in this laboratory. Rose (11) has shown that invert sugar may be determined in the presence of sucrose by the use of picric acid and sodium carbonate according to the method of Lewis and Benedict. We have observed that the use of the newer method of Benedict and Osterberg also makes possible the determination of small amounts of glucose in the presence of sucrose. The sucrose fed was twice precipitated from alcohol and showed on analysis in the polarimeter a purity of 99.98 per cent.

The subjects were healthy men and women, students in the laboratory. The experiments were carried out on fasting subjects, who had received no food since the evening meal of the previous day. In the earlier experiments the meal consisted of the whites of three eggs cooked over water till the protein was partly coagulated. Since in the experiments with egg white alone it was found that egg white contained a small amount of glucose for which correction had to be made, in later experiments 50 grams of cottage cheese were fed. Cottage cheese as purchased was repeatedly triturated with water (10 to 15 times) and drained through cheese cloth until the wash water gave no appreciable reduction with Fehling's solution.

Fifteen minutes after the protein meal the Rehfuss tube was swallowed, and after 5 minutes 5 grams of sucrose in 200 cc. of water were fed through the tube. In a few experiments 100 cc. of distilled water were fed before the ingestion of the sucrose and, as a control on the glucose content of the meal, a sample of gastric contents was removed before the ingestion of the sucrose. After the introduction of the sugar, samples were removed at 20-minute intervals for analysis. Acidity was determined by titration with standard sodium hydroxide with the

use of dimethylaminoazobenzene and phenolphthalein respectively as indicators. An aliquot was neutralized immediately and the protein precipitated by the addition of the picrate-picric acid reagent with which the sample was diluted to 25 cc. Glucose was then determined by the Benedict-Osterberg modification of the Lewis-Benedict method, pure glucose being used as standard. These results represent the amount of reducing sugar (calculated as glucose) present in the sample as removed from the stomach. A second aliquot was incubated at 37° for 2 hours and the reducing sugar determined as before. The results in this case show the amount of inversion possible if the gastric juice were afforded opportunity for more prolonged action on the sucrose. To a third portion, hydrochloric acid was added to give a final concentration of 1.0 per cent, the mixture hydrolyzed by boiling for one hour, and the reducing sugar determined after neutralization of the acid. Boiling in acid solution should completely invert the sucrose and the subsequent determination of reducing sugar should then show total carbohydrate, sucrose plus invert sugar originally present. In all cases allowance was made for the retardation of the development of the color occasioned by the presence of the chlorides formed by neutralization of the acid (12).

Since regurgitation from the intestine at intervals is now known to accompany gastric digestion normally, the presence of a sucrase was tested for in the gastric contents. A sample of gastric contents was withdrawn before the introduction of the sucrose, and the acidity neutralized. Sucrose was added and the mixture incubated for 2 hours. Any inversion in this case would indicate the presence of sucrase since the other factor which might cause hydrolysis, the acid, was eliminated. In none of the tests was there any hydrolysis, thus indicating the absence of any active sucrase. It may be noted that in 1902 Widdicombe (13) reported the presence of active sucrase in the gastric juice of dogs and pigs. Lusk (14), however, in 1904 repeated these experiments with dogs and was unable to corroborate the findings of Widdicombe. He concluded that to hydrochloric acid alone is due the action of gastric juice on sucrose.

DISCUSSION

Hydrolysis of sucrose by gastric juice in vivo. In tables 1 and 2 are presented typical data obtained. Other experiments on different subjects have given similar results. In the experiments where the egg white meal (table 1) was ingested, there is apparently considerable

hydrolysis, more than might be expected from the action of the acid alone. However, if these figures are corrected for the glucose present in the stomach after ingestion of an egg white meal without added

TABLE 1

The hydrolysis of cane sugar in the stomach following ingestion of egg whites

SUBJECT	TIME AFTER INGESTION	FREE ACIDITY	TOTAL ACIDITY	REDUCING SUGAR PER 5 CC. GASTRIC CONTENTS	REDUCING SUGAR PER 5 CC. AFTER INCUBATION	TOTAL SUGAR PER 5 CC. AFTER ACID HYDROLYSIS	INVERSION IN STOMACH	INVERSION AFTER INCUBATION
	mins	*cc.* $\frac{N}{10}$ *acid*	*cc.* $\frac{N}{10}$ *acid*	*mgm.*	*mgm.*	*mgm.*	*per cent*	*per cent*
J. J.	20	1.4	29.3	3.5	4.3	34.5	10.2	12.3
	40	18.6	24.3	1.8	2.0	11.1	16.2	18.0
	60	18.0	28.6	1.1	1.5	6.0	18.6	25.2
H.	20	14.1	31.4	2.2	4.0	26.8	8.3	14.9
	40	21.4	34.3	1.7	2.0	9.3	18.1	21.5
	60	31.4	42.0	1.1	1.4	7.7	14.3	18.2
M.	20	2.8	47.9	4.0	2.7	36.5	11.0	7.4
	40	17.9	54.3	3.8	3.1	32.5	11.5	9.5
	60	34.6	60.3	3.1	3.2	19.3	16.0	16.6
C. B.	20	25.0	49.0	6.0	7.1	53.5	11.2	13.3
	40	34.0	84.0	5.7	5.9	36.5	15.6	16.2
	60	36.0	75.0	4.1	6.1	20.5	19.8	29.8
W. P. B.	20	25.0	33.0	3.1	3.9	49.5	6.3	8.0
	40	42.0	60.0	2.0	3.2	17.3	11.6	18.5
	60	41.0	56.0		0.8	4.0		19.0
				1.9*				
C. B.†	20	10.9	27.6	2.5	2.4			
	40	50.8	70.8	1.7	1.9			
	60	54.4	77.6	1.9	2.1			

* Sample taken before sucrose administration.

† In this experiment no sucrose was ingested. The gastric contents were removed at periods indicated in the table, following the ingestion of egg white. The experiment serves as a control on the amount of reducing sugar in the egg white meal.

sucrose (subjects W. P. B. and C. B., table 1), the hydrolysis observed is comparable with that obtained after the ingestion of cottage cheese (table 2), a degree of hydrolysis hardly of significance in the digestive process. It is realized that it is difficult to apply these corrections

accurately because of the marked individual variations in the velocity with which the sugars are discharged from the stomach (cf. subjects C. B. and W. P. B., table 1; C. B. and J. B. H., table 2). The uncorrected figures in table 1, however, may be taken to represent maximum values for the inversion of sucrose in the stomach. While the percentage inversion may appear significant in the samples removed after an hour, it must be borne in mind that it is in reality of slight consequence in most cases because of the small quantity of sugar remaining in the stomach at that time.

After incubation for 2 hours at body temperature, there was observed a hydrolysis closely parallel to that effected by hydrochloric acid alone

TABLE 2

The hydrolysis of cane sugar in the stomach following ingestion of cottage cheese

SUBJECT	TIME AFTER INGESTION	FREE ACIDITY	TOTAL ACIDITY	REDUCING SUGAR PER 5 CC. GASTRIC CONTENTS	REDUCING SUGAR PER 5 CC. AFTER INCUBATION	TOTAL SUGAR PER 5 CC. AFTER ACID HYDROLYSIS	INVERSION IN STOMACH	INVERSION AFTER INCUBATION
	mins	*cc.* $\frac{N}{10}$ *acid*	*cc.* $\frac{N}{10}$ *acid*	*mgm.*	*mgm.*	*mgm.*	*per cent*	*per cent*
C. B....	20	34.3	42.9	1.7	8.0	57.5	3.0	13.9
	40	44.3	57.2	4.0	12.3	63.0	6.3	19.5
	60	37.2	45.8		2.9	17.9		16.2
J. S.....	20	26.5	37.9	2.2	10.4	100.0	2.2	10.4
J. B. H.	20	17.2	20.6	0.6	2.1	31.8	1.9	6.6
	40	20.6	27.2	1.5	1.7	14.0	8.2	12.1
	60	25.7		0.8	1.0	3.6	22.2	28.0

in physiological concentrations. The results of these experiments are in harmony with those of Ferris and Lusk (8). It is evident that the acid normally present in gastric juice may effect a considerable degree of hydrolysis of sucrose provided sufficient time is allowed for its action in the stomach. The carbohydrates, however, as shown by the figures for total sugar after hydrolysis, have almost completely left the stomach at the end of an hour.

The action of gastric juice on sucrose in vitro. In order to confirm and extend the findings in the feeding experiments already described, a series of tests was carried out on the action of gastric juice on sucrose in vitro (table 3). The egg white meal was fed in every case, and only one sample of gastric contents withdrawn. This was removed 40 minutes

after the meal, which seemed to be the average time of maximum acidity under the conditions of these experiments. The acidity of the juice was determined in one aliquot, while a second was analyzed for reducing

TABLE 3

The hydrolysis of sucrose by gastric juice in vitro*

SUBJECT	GASTRIC JUICE	SUCROSE ADDED	PERIOD OF INCUBATION	FREE ACIDITY	TOTAL ACIDITY	REDUCING SUGAR PER 5 CC. GASTRIC JUICE	NOTES
	cc.	*mgm.*	*hours*	*cc.* $\frac{N}{10}$ *acid*	*cc.* $\frac{N}{10}$ *acid*	*mgm.*	
M. L. B.	10	50	2	22	34	2.6	Gastric juice as taken from stomach
	10	50	2	22	34	2.6	Sample boiled before addition of sucrose
	10	50	2	0	0	1.1†	Neutralized and boiled before addition of sucrose
W. P. B.	10	50	2	20	35	4.0	Gastric juice as taken from stomach
	10	50	2	20	35	3.9	Boiled before addition of sucrose
	10	50	2	0	0	3.8	Neutralized before addition of sucrose
	10	50	2	0	0	3.7†	Boiled and neutralized before addition of sucrose
F. H. T.	10	50	2	44	68	7.5	Gastric juice as taken from stomach
	10	50	2	44	68	7.5	Boiled before addition of sucrose
	10	50	2	0	0	3.0	Neutralized before addition of sucrose
	10	50	2	0	0	4.3†	Boiled and neutralized before addition of sucrose
	10	0	0	44	68	4.1†	Without added sucrose

* The egg-white meal was used.

† The last test with each subject gives the correction to be applied for the glucose content of the meal.

sugar. To each of a series of aliquots of 10 cc. each, was added 0.050 gram of sucrose. The first was incubated 2 hours at 37°. This is comparable to similar tests in the feeding experiments and gave similar results, as may be seen from a comparison of the values obtained with

those of the incubated samples in tables 1 and 2. A second aliquot was heated to boiling and cooled before the addition of the sucrose, and then incubated for 2 hours. This procedure should destroy any active sucrase which might be present and inversion observed should be due solely to the action of the acid. The third aliquot was not heated, but was neutralized before the addition of the sucrose, and then incubated. If any hydrolysis occurred in this case, it would be presumably due to factors other than the acidity and would indicate the presence of enzymes. The fourth aliquot was both boiled and neutralized before the sucrose was added. This was run as a control on the amount of reducing sugar originally present and no inversion was to be expected.

The experiments failed to demonstrate the presence in the gastric juice of any agent active in the inversion of sucrose other than the hydrochloric acid. This is best shown by the experiments with F. H. T. (table 3) in which the acidity developed in the gastric contents was higher than in other cases. Boiling unneutralized gastric contents did not affect their power to invert sucrose, while neutralization alone resulted in complete loss of power to hydrolyze the sugar. This supports the view that no enzyme action is concerned and that the presence of "free" acid is necessary for inversion. The experiments further demonstrated that if sucrase is carried into the stomach by intestinal regurgitation, it does not remain active long enough to cause any appreciable hydrolysis in the stomach. The in vitro studies confirm our experiments on hydrolysis in vivo and are in agreement with the conclusions reached by Lusk (14) in his experiments on dogs.

SUMMARY

The inversion of sucrose in the human stomach has been studied by the fractional method of analysis of gastric contents. Any hydrolysis of sucrose which takes place in the stomach may be explained solely by the action of the hydrochloric acid present, and varies with the concentration of the acid. The inversion under normal conditions in man is too slight to be of significance since carbohydrates leave the stomach too rapidly to permit of prolonged action of the acid. It has not been possible to demonstrate the presence of an active sucrase in gastric contents in spite of the fact that intestinal regurgitation had occurred in many cases as evidenced by the presence of bile.

BIBLIOGRAPHY

(1) RUBNER: Disquis de sachar. cannae in tractu cibario mutationibus, diss. inaug., Breslau, 1859; cited by VOIT: Zeitschr. Biol., 1891, xxviii, 268.
(2) BERNARD: Physiol. exper., Paris, 1856, ii, 402.
(3) HOPPE: Arch. pathol. Anat.,1856, x, 144.
(4) KULZ: Beitr. Path. Therap. des Diabetes Mellitus, Marburg, 1874, 147; cited by VOIT: Zeitschr. Biol., 1891, xxviii, 286.
(5) WORM-MÜLLER: Arch. gesammt. Physiol., 1884, xxxiv, 586.
(6) SEEGEN: Arch. gesammt. Physiol., 1887, xl, 39.
(7) VOIT: Zeitschr. Biol., 1891, xxviii, 268.
(8) FERRIS AND LUSK: This Journal, 1898, i, 277.
(9) BOLDYREFF: Quart. Journ. Exper. Physiol., 1914, viii, 1. SPENCER, MEYER, REHFUSS AND HAWK: This Journal, 1916, xxxix, 459.
(10) OKEY: Journ. Biol. Chem., 1919, xxxix, 149.
(11) ROSE: Proc. Soc. Exper. Biol. Med., 1917–18, xv, 16; Journ. Biol. Chem., 1921, xlvi, 529.
(12) OKEY: Journ. Biol. Chem., xxxviii, 1919, 33.
(13) WIDDICOMBE: Journ. Physiol., 1902, xxviii, 175.
(14) LUSK: Proc. Amer. Physiol. Soc., This Journal, 1904, x, p. xxi.

THE MYOGENIC NATURE OF THE RHYTHMIC CONTRACTIONS OF THE INTESTINE

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There are few statements in physiology upon which opinion is more unanimous than the one to the effect that the rhythmic contractions of the bowel are neurogenic, and due to impulses coming from Auerbach's plexus. We find that statement in almost all of the textbooks and articles which we have consulted in the last eight years. Such unanimity is rather surprising, but it is still more surprising when we learn that practically all the research work done on the subject points clearly in the opposite direction; and it is even more surprising when we learn that Magnus, whose early negative results are so generally quoted and accepted, later obtained positive results which quite upset his preliminary deductions. Unfortunately, it is easier for writers to quote from the English abstracts of Magnus' first two papers (1) than to wade through the seventy-one pages of German in the fifth Mitteilung (2) which records his success in getting denervated muscle to contract rhythmically.

In his first paper, Magnus describes the technic of using excised segments of intestine in Locke's solution and records a number of preliminary studies. In his second paper, he shows that the longitudinal muscle can be pulled off of the circular, carrying with it practically all of Auerbach's plexus. That is due to the fact that the nerve cells lie in grooves and deep recesses in the longitudinal layer. He studied his preparations after careful staining and found that only small fragments

[1] This work was made possible through the kindness of: Mr. T. E. Bibbins, Dr. W. B. Coffey, Mr. Mortimer Fleischhacker, Dr. John Gallwey, Mr. E. Clemens Horst, Dr. N. W. Jones, Mr. G. A. Mattern, Dr. Langley Porter, Dr. H. A. L Ryfkogel, and Dr. A. J. Zobel, who paid Miss Mahoney's salary during the past year.

of the plexus remained adherent to the circular layer. These little pieces were so widely scattered and so badly damaged that he felt they could be disregarded. As the longitudinal muscle with the plexus attached contracted rhythmically and the circular did not, he was satisfied that the beat was neurogenic. Later, in his fourth paper (3), he showed that the active, plexus-containing strips and the quiet denervated ones reacted differently to electric stimuli. Some difficulties were met with in this study which it seems to us should have caused Magnus to doubt the sureness of his ground, but his troubles began in earnest when he started studying the effects of drugs on the segments. He was delighted at first to find that some of the drugs brought out distinct differences in the behavior of the plexus-free and plexus-containing segments, but later he discovered that certain substances such as barium chloride, strophanthin and pilocarpin would bring out rhythmic contractions even in the denervated segments. This led him to the conclusion that, although the preparations of circular muscle which he had been using were denervated sufficiently for most types of physiologic study, they were not denervated sufficiently for pharmacologic work. In order to remove all traces of the plexus, he seared the outer surface of the circular muscle with crystals of nitrate of silver; and when strips treated in this way failed to contract rhythmically even in the presence of the above-mentioned drugs, he felt that he had gotten rid of all traces of the plexus. Unfortunately for his peace of mind, he found next that physostigmin would bring out beautifully rhythmic contractions even in the partly charred strips which he was sure were plexus-free. These contractions are shown in figure 1, *A*, copied from his paper. As he could go no farther in the matter of denervation, and as physostigmin brought out rhythmic contractions in strips which reacted in a typically "denervated" way to other tests, Magnus had to admit that the contractions were myogenic. He found, moreover, that physostigmin acts in the same way on the denervated muscles of sipunculus nudus (4). In that animal there can be no doubt about the completeness of the denervation because the ventral nerve cord is easily visible and can be dissected off in its entirety.

In 1914 Gunn and Underhill (5) repeated this work of Magnus, using a better technic. They felt that they could cut down the severity of the shock of dissection by tearing the strips apart under iced Locke's solution, which acted as an anesthetic. As they had good reasons for believing that the silver nitrate used in searing the outer surface of the circular layer would diffuse into the tissue and damage the muscle through-

out the strip, they avoided using it by taking for their experiments only a few fibers from the mucous membrane side. In this way they obtained muscle which certainly would seem to have been plexus-free, and which contracted rhythmically without the help of any drugs (see fig. 1, *B*, copied from their article). They were impressed also with the fact which has always interested us and that is that the segments continue to contract rhythmically for 4 or 5 days after removal from the animal. We have records showing that canine and human intestinal segments will contract even more regularly on the third day than on the first. The rate of contraction in the rabbit's small intestine is sometimes even faster and more regular on the second day than on the first (6). As it has been shown experimentally that some sympathetic ganglia fail to conduct stimuli within a few minutes after the stoppage of the circulation, it does not seem reasonable to assume that the function of the intestinal ganglia can *improve* after 48 hours of anemia. A similar persistence over several days of the rhythmic contractions in the retractor penis of the dog and in the cat's bladder convinced De Zilwa (7) and Stewart (8) that the underlying mechanism must reside in the muscle.

Another interesting observation of Gunn and Underhill was that the ability of the muscle to contract rhythmically; its ability to respond to drugs like atropin, pilocarpin and adrenalin, which are supposed to act on nerve endings; and its ability to respond to barium chloride which is supposed to act on muscle, were all lost together on the fifth day when the tissue apparently died. We have repeated these experiments and have also failed to obtain any evidence of nerve endings dying on one day and muscle on another.

Under the circumstances, Gunn and Underhill felt that there could be no doubt about the myogenic origin of the rhythmic contractions. Bayliss and Starling (9) had come to a similar conclusion many years before. They found that while cocain, applied to the surface of the gut, abolished the myenteric reflex, it actually improved the amplitude and regularity of the rhythmic contractions. As nicotin had the same effect when injected repeatedly and in large doses, they had good reasons for believing that they had paralyzed the plexus; that the myenteric reflex is due to the nerves; and that the rhythmic contractions are due to the muscle. Although it is best not to put too much trust in physiologic deductions based upon the assumed selective actions of poisons, it does seem to us that this evidence must be faced. Certainly we cannot go on as we have been doing, quoting this work to show that the prog-

ress of food through the bowel is based on the nervous mechanism, and Magnus' early work to show that the rhythmic contractions are also based upon it. A brief analysis of the problem will show that if Bayliss and Starling were right Magnus must have been wrong, and vice versa.

Recently Hammett (10) has shown that excised segments of rats' bowels will continue to contract rhythmically in a 1:4000 solution of cocain; and we have found that similar segments of rabbits' ileum will continue beating in 1:360 novocain solution. Fulton (11) has shown beautifully that novocain acts on the "intermediate substance" between nerve and muscle, and that this substance is apparently to be found in the nuclei of the muscle cells. He shows that novocain has little effect on the heart beats of the embryo chick, either before or after nerves grow into it. What it does is to block the nerve endings, so that stimulation of the vagus is no longer effective. This pharmacologic evidence all points again to the myogenic origin of the intestinal contractions.

When one remembers how common rhythmical processes are in nature, it is hard to understand why physiologists tend so often to ascribe those observed in muscle to that highly specialized organ, the nervous system. Thus Ostwald (12) showed that curves closely resembling those traced by a frog's heart can be produced simply by attaching an ordinary tambour to a flask in which hydrochloric acid is acting on chromium, with the evolution of hydrogen. Rhythmic changes take place in the metal which make it alternately resistant and subject to attack by the acid (see fig. 1, *c*, copied from Ostwald's article). Similar rhythmic changes occur in the rate of catalytic decomposition of hydrogen peroxide in contact with mercury, owing to the alternate formation and dissolution of a surface film of "peroxidate." In both of these reactions electricity plays a large part, much as it does in the animal body. Lillie (13) has been able to produce rhythmic "action currents" by putting two piano wires in nitric acid and connecting them to a galvanometer, and we have observed similar currents arising in calomel-electrodes that have been used too long.

When water drips at regular intervals from a reservoir we have a rhythmic response to a constant force, and Forbes and Gregg (14) have remarked upon several instances of this type of reaction in the body. Quincke (15) produced rhythmic alterations in the shape of an air bubble, trapped under water, and placed in the path of a fine stream of alcohol. The pulsation of the bubble closely resembles that of a heart, and is due to alterations in the surface tension. Passing onward from

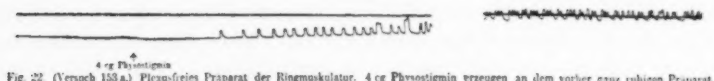
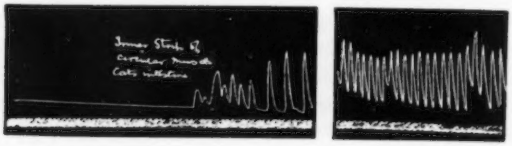


Fig. 22. (Versuch 153 a.) Plexusfreies Präparat der Ringmuskulatur. 4 cg Physostigmin erzeugen an dem vorher ganz ruhigen Präparat rhythmische Spontanbewegungen mit allmählicher Tonussteigerung.

A

Fig. 23. (Versuch 156 A.) Plexusfreies Präparat der Ringmuskulatur. Das Präpart steht seit 16 Min. unter der Einwirkung von 5 cg Physostigmin, ohne Bewegungen zu zeigen. Auf 0.2 mg Strophanthin Thoms zunächst glatte, charakteristische Strophantinkontraktion, darauf Einsetzen rhythmischer (Physostigmin-)Bewegungen.

B



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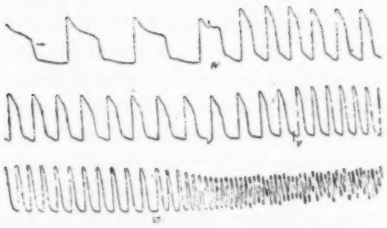

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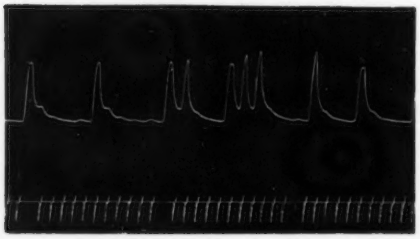

Fig. 1. *A*, Copy of Magnus' illustration showing rhythmic contractions in plexus-free preparations. *B*, Copy of Gunn and Underhill's illustration showing the same thing. *C*, Copy of Ostwald's illustration showing rhythmic changes in a relatively simple inorganic chemical reaction. Note the increase in rate with a rise in temperature. *D*, Rhythmic contractions obtained from strips of muscle removed from the inner face of the circular layer of the dog's intestine, the longitudinal layer, with the plexus, having previously been removed. The time record represents 5-second intervals.

these relatively simple chemical and physical phenomena, we find that Bose (16) has obtained beautifully rhythmic tracings of the contractions of the leaflets of certain plants. A little higher in the scale, we find animal tissues, not yet muscular in structure, but possessing the power of rhythmic contraction. We need only mention cilia and the tails of spermatozoa.

The heart beat seems to originate in muscle or muscle-like tissue except in the Limulus, a very primitive type of crab; and even in that animal Garrey (17) has shown that the denervated muscle will contract rhythmically if put into M/2 NaCl. Gaskell (18) found that the heart of a leech can be removed entirely from the nervous influences without stopping its activity. The heart of a tunicate will continue to beat when separated from its ganglion and even when removed from the body (19). Luchsinger (20) found that the veins in a bat's ear will contract rhythmically if they are perfused even as late as 50 hours after death. The heart of an embryo chick beats after 29 hours of incubation, although nerve cells do not reach it until the sixth day (21); and even the isolated heart muscle cells in tissue cultures have been observed to beat (22). The same thing was seen by Lewis and Lewis (23) in cultures of smooth muscle and by Lewis (24) in cultures of skeletal muscle. The retractor penis of the dog is made up of smooth muscle; it has no ganglia (25) and yet it contracts rhythmically when excised (26). Loeb (27) has shown, moreover, that even striated muscle will contract rhythmically in the proper concentration of NaCl. Nice and Neill (28) have recently called attention to rhythmic changes in diaphragm muscle. Hydromedusa stops pulsating when the nerve net is excised and this was supposed to prove the nervous origin of the beat until Loeb (29) showed that the muscle will start up again if the concentration of the seawater is changed. Romanes (30) found, moreover, that the nerveless centers of other types of jellyfish will sometimes beat in seawater after the shock of the operation has worn off.

This review of the literature shows that rhythmic contraction is a property inherent in muscle and muscle-like tissues; and there is good reason to believe that it is brought about rather simply as the result of recurring cycles of chemical activity. Similar metabolic cycles might be present just as well in nervous tissue; and there is no doubt that in some situations, as in the bell of the medusa or the heart of Limulus, the normal pacemaker is to be found in ganglion cells. The only article that we can find in support of the idea of a neurogenic origin for the intestinal contractions is that by Yanase (31) who observed peristalsis

in embryo guinea pigs only after the 26th day, when the myenteric plexus and the longitudinal muscle appear. This, however, is negative evidence which may be objected to for a number of reasons.

Experimental work. Remembering that the dog's intestine often contracts better on the second or third day after excision, we thought it well to test the rhythmicity of circular muscle, stripped away from the longitudinal, 24 hours after the bowel had been placed in the icebox. Following Gunn and Underhill, we used only the inner half of the circular layer lying next to the mucous membrane which was also stripped off. We were able to get rhythmic contractions from such muscle which we felt sure had been freed from all traces of the nerve net. In some cases we were able to show with the microscope that considerable tissue had been left between the plexus and the muscle fibers removed for the experiment. It was not difficult to get rhythmic contractions from such segments after adding a trace of barium to the Locke's solution. Without the barium we had to wait sometimes for 6 or 7 hours before contractions appeared.

Our recent work with delicate galvanometers has shown that strong rhythmic action currents are being developed in segments of intestine which, so far as the eye can see, are absolutely quiet (32). This observation brings up some interesting questions. It suggests that rhythmic chemical processes are continually going on in the muscle; processes which, from time to time, give rise to the physical contractions. The presence of a little barium or physostigmin seems to act like the clutch in an automobile which causes the energy of the engine to appear at the back wheels as motion. Similarly in the heart, Mines (33) found that the withdrawal of calcium stopped the beats but not the action currents. De Meyer (34) thinks that in the heart there is no relation between the beat and the intensity of the action current because the current remained unchanged when the heart was stopped by clamping the aorta. We have found that splanchnic stimulation, or the painting on of adrenalin, will stop the intestinal contractions and still leave the currents. It is clear, then, that we cannot always say, when a muscle is not beating, that it has lost its source of rhythmic stimuli. In the language of the simile, the engine may still be running but the clutch may be out. Some may still suspect that the rhythmic engine resides in the nerve net but that seems unlikely on account of the intensity of the currents produced. Our impression is that current intensities are more or less proportional to the volume of muscle involved, i.e., the currents in a dog are much more powerful than those in a rabbit. Yet there is remarkably

little difference sometimes between the action currents of an immobile and an actively segmenting bowel. A glance at figure 2 will show that the intensity of the action currents in an excised segment of rabbit's ileum, rendered absolutely immobile by adrenalin, is about four-tenths of the intensity of the currents in the active bowel. In some experiments it was nearer six-tenths. If the currents of the inactive bowel were generated in the tiny nerve net and those of the active bowel in the net and muscle together, we would expect a much larger difference. In passing, it is of interest to note that this adrenalin which stopped the rhythmic movements of the muscle increased the rate of the rhythmic action currents from 10.5 to 14 per minute.

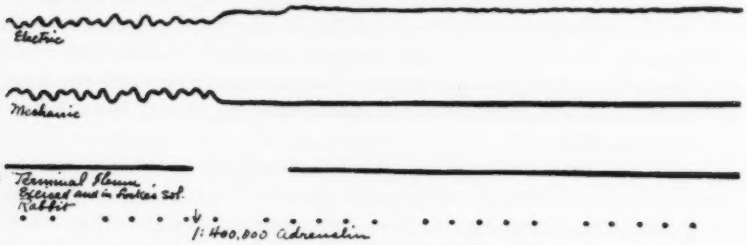

Fig. 2. Upper record shows the electric changes, the lower shows the mechanical contractions. The break in the signal line shows when adrenalin was added. The time record represents 10-second intervals. A segment of rabbit's ileum was suspended in a beaker containing 400 cc. of warm aerated Locke's solution. The currents were led off from specially designed calomel electrodes. The contractions of the gut were transmitted by tambours to the photographic device described elsewhere.

In closing, it should be pointed out that the question of the neurogenic or myogenic origin of the contractions is a more or less academic one because under normal conditions the muscle and nerve fibers are intimately associated and are designed to work together. There is no question but that in most cases the muscle works better for the presence of its nervous plexus. The immediate effect of the removal of the nerves is shock and spastic paralysis; and, when that is recovered from, we cannot expect the tissue to work quite so well as it did normally. The conduction from muscle bundle to muscle bundle must remain impaired.

Our reasons for reviewing the subject so at length are twofold: first, to point out certain errors which have crept into the literature; and second, to call attention to the importance of studying the muscle

as well as the nervous tissue. We feel that the tendency to push the explanation for vital phenomena out of the organs in which they might be studied and into elusive little ganglion cells is often unfortunate, and conducive more to stagnation of thought than to advancement of knowledge.

SUMMARY

Most writers quote Magnus' early work to the effect that the rhythmic contractions of the intestine are neurogenic in origin. Few seem to know that in later experiments he was able to get good contractions from well denervated muscle.

The positive results were subsequently confirmed by Gunn and Underhill, and now by the writers. They are in agreement also with the findings of Bayliss and Starling.

It is shown now, moreover, that even if a bit of intestinal muscle fails to beat, it does not follow necessarily that it has lost its source of rhythmic stimuli, because wave-like action currents can, in many cases, still be demonstrated. These currents are so nearly equal in intensity to those observed in active segments that it can hardly be argued that they arise in the nervous tissue, the mass of which is small.

A review of the literature on rhythmic processes in nature shows that there is no need for looking beyond the muscle for the source of the rhythm.

BIBLIOGRAPHY

(1) Magnus: Arch. f. d. gesammt. Physiol., 1904, cii, 123; ibid., 349.
(2) Magnus: Arch. f. d. gesammt. Physiol., 1905, cviii, 1.
(3) Magnus: Arch. f. d. gesammt. Physiol., 1904, ciii, 525.
(4) Magnus: Arch. f. Exper. Pathol., 1903, i, 86.
(5) Gunn and Underhill: Quart. Journ. Physiol., 1914, viii, 275.
(6) Alvarez: This Journal, 1918, xlv, 342.
(7) De Zilwa: Journ. Physiol., 1901, xxvii, 200.
(8) Stewart: This Journal, 1900, iv, 185.
(9) Bayliss and Starling: Journ. Physiol., 1899, xxiv, 99.
(10) Hammett: This Journal, 1921, lv, 414.
(11) Fulton: This Journal, 1921, lvii, 163.
(12) Ostwald: Zeitschr. f. physikal. Chem., 1900, xxxv, 33.
(13) Lillie: Journ. Gen. Physiol., 1920, iii, 115.
(14) Forbes and Gregg: This Journal, 1915, xxxix, 172.
(15) Quincke: Quoted from Loeb, Comparative physiology of the brain, New York, 1900, 22.
(16) Bose: Researches on the irritability of plants, London, 1913.
(17) Garrey: Journ. Gen. Physiol., 1920, iii, 41.

(18) GASKELL: Journ. Gen. Physiol., 1920, ii, 73.
(19) DAY: Journ. Exper. Zoöl., 1921, xxxiv, 45.
(20) LUCHSINGER: Arch. f. d. gesammt. Physiol., 1881, xxvi, 452.
(21) HIS: Abth. math. phys. Cl. sächs Gesell. Wissen, 1893, xviii, 1; Quoted from FULTON: This Journal, 1921, lvii, 163; VERZAR; Arch. f. d. gesammt. Physiol., 1914, clviii, 419.
(22) BURROWS: Science, 1912, xxxvi, 90; LAKE: Journ. Physiol., 1916, l, 364.
(23) LEWIS AND LEWIS: This Journal, 1917, xliv, 67.
(24) LEWIS: This Journal, 1915, xxxviii, 153.
(25) FLETCHER: Journ. Physiol., 1898, xxii, xxxvii, xl; FISHER: Anat. Record, 1917, xiii, 69.
(26) DE ZILWA: Journ. Physiol., 1901, xxvii, 200; EDMUNDS: Journ. Pharm. Exper. Therap., 1920, xv, 201.
(27) LOEB: The dynamics of living matter, New York, 1906; MINES: Journ. Physiol., 1908, xxxvii, 408.
(28) NICE AND NEILL: This Journal, 1921, lvii, 171.
(29) LOEB: The comparative physiology of the brain, New York, 1900, 18.
(30) ROMANES: Phil. Trans. Roy. Soc., 1876, clxvi, 269.
(31) YANASE: Arch. f. d. gesammt. Physiol., 1907, lxvii, 345.
(32) ALVAREZ: This Journal, 1921-22, lviii, 476.
(33) MINES: Journ. Physiol., 1913, xlvi, 224.
(34) DE MEYER: Arch. int. d. Physiol., 1910, x, 100.

EFFECTS OF THE GALVANIC CURRENT ON SEGMENTS OF INTESTINE

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Alvarez and Starkweather (1) showed in 1918 that there is a gradient of metabolism in the muscle of the small intestine from the duodenum to the ileum. Per unit of weight and time the muscle from the duodenum gives off more CO_2 than the ileum. Similar gradients along the polar axes of lower forms of life have been shown to be associated with gradients in electrical potential (2); that is, the head end of the animal is negative (through the galvanometer) to the tail end. These differences in electrical potential cause the animal to resemble in a way a small magnet, the positive pole of which is at the head end and the negative pole at the tail. According to Child, this electrical polarity can account in part for the fact that such organisms, when placed in water through which a current is flowing, generally take up positions in the lines of force with their head ends toward the cathode. Some have felt that these positions are assumed because they give the animals least discomfort, but Loeb and others have shown that this so-called galvanotropism is due simply to the unequal or dissimilar effects of the entering and emerging currents on the muscles of the animal.

It occurred to us that inasmuch as the intestinal segments show a polarity, similar to that found by zoölogists in small water organisms, they might show a somewhat similar behavior when placed in the path of an electric current. We have presented elsewhere some evidence that there is an electrical gradient down the bowel from the duodenum to ileum, orad segments being negative to caudad ones (3). Alvarez

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and Starkweather (4) showed, moreover, as was to be expected, that conduction is better down the bowel with the gradient than orad, against that gradient. It seemed worth investigating, then, to see whether there would be any difference in the effects of a current traveling caudad down the gradient and one traveling orad, against it.

In order to show differences in the effects of ascending and descending currents, two segments were put into a glass tube 3 cm. in diameter and 18 cm. long. The lower end was closed by a rubber cork pierced by a copper wire and a small tube for the air supply. Two segments of rabbit's small intestine, 3 cm. long, were suspended in this tube and fastened to heart levers so that they would write one above the other on a smoked drum. The tube was filled with Locke's solution aerated from below, and kept warm by immersion in a large water bath. Another copper wire dipped into the solution at the upper end of the tube. The current was supplied ordinarily by two dry cells in series, sufficient to produce definite effects on the bowel.

Sometimes the two segments were placed both with the cephalad ends up; sometimes one was placed with the cephalad end up and the other with it down. The direction of the current was then changed from time to time and the results were noted (see fig. 1). Although the segments seemed to writhe more actively, these movements were integrated in such a way as to appear on the drum as a lessening of amplitude with a slightly increased tone. No change in the rate of contractions could be detected; and up to this point the work lent no support to the gradient theory.

In the second set of experiments, segments of rabbit's intestine about 10 cm. long were put into a flat dish full of warm Locke's solution and the current from two dry cells was led in through copper wires. When the direction of the current was perpendicular to the long axis of the bowel the segment exhibited powerful writhing movements which showed a striking resemblance to those of an angle worm under similar circumstances. As was to be expected, the muscle on the cathodal side was stimulated so much more than that on the anodal that the loop was made to bend with its two cut ends approaching the cathode. In so doing it often was caused to turn over on account of the inextensible mesenterial attachment on one side. This would often bring another stretch of muscle onto the cathode side and more tumbling movements would result. When the segments were placed with the long axes in the path of current flow, the muscle on the end toward the

cathode was stimulated most, with the result that in a typical experiment powerful waves began at that end and coursed to the other. The striking thing was that these waves could be sent alternately in the orad and caudad direction through the segment, simply by reversing the direction of the current.

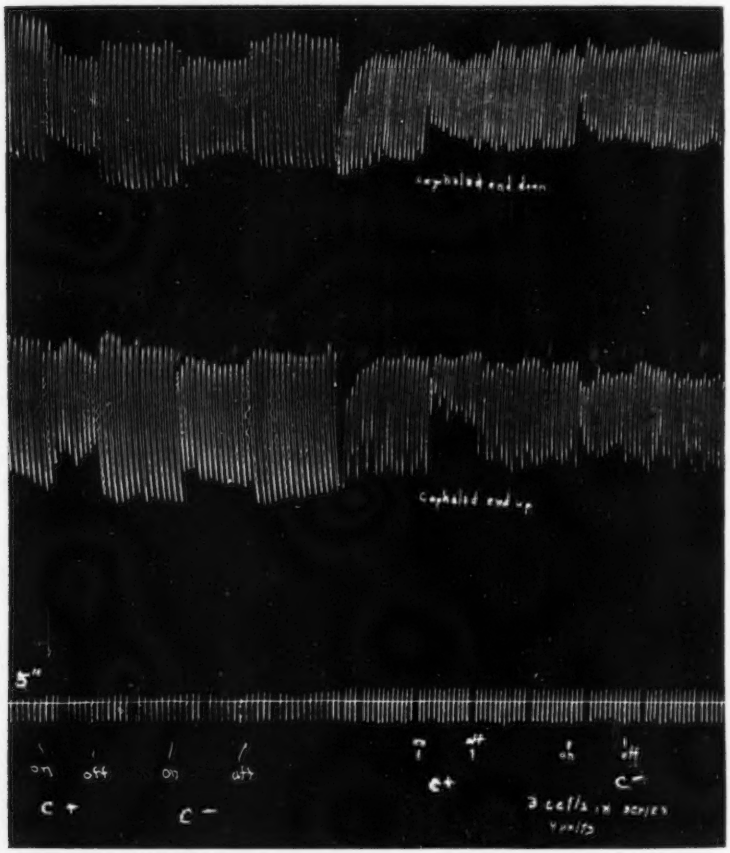

Fig. 1. Tracings from excised rabbit's jejunum (left) and ileum (right) beating in a long tube of oxygenated Locke's solution. On the left, the cephalad ends were up; on the right, one segment had its cephalad end down and the other had it up. $C+$ means that the current entered at the upper end of the tube; $C-$ that it entered below.

It seems to us that these experiments lend support to the gradient idea of peristalsis because they show that the direction of the intestinal waves can be reversed by an electric current which reverses the gradient of muscular activity. Our work lends support also to the idea often expressed by Loeb, Parker, Garrey and others, that a worm or other lowly animal does not writhe about in certain ways under a stimulus because it is in pain and is hunting for a comfortable position, but because certain muscle groups are predominately affected by the stimulus. If we are to ascribe intelligence to the worm, we must look for it also in the excised bowel because that not only writhes in a wonderfully life-like manner when placed in the path of the electric current, but it shows a definite tropism.

SUMMARY

There is a metabolic gradient down the bowel from duodenum to ileum. Associated with this, there appears to be a gradient of electrical potential: in other words, segments of bowel are polarized as are many of the worms and plants.

When placed transversely across the path of a galvanic current short sections of rabbit's bowel showed a galvanotropism due to a greater stimulation of the longitudinal muscles on the cathodal side. When a segment was placed longitudinally in the path of the current peristaltic waves began at the cathodal end and ran to the anodal. The direction of peristalsis could be reversed by reversing the current.

When such segments were made to pull on a lever attached to one end, no difference was observed in the graphs obtained with ascending and descending currents.

BIBLIOGRAPHY

(1) ALVAREZ AND STARKWEATHER: This Journal, 1918, xlvi, 186.
(2) CHILD: Biol. Bull., 1920, xxxix, 147.
HYMAN: Science, 1918, xlviii, 518.
(3) ALVAREZ AND MAHONEY: This Journal, 1921, lviii, 476.
(4) ALVAREZ AND STARKWEATHER: This Journal, 1919, l, 252.

THE ACTIVATION OF THE GLANDULAR STOMACH OF THE FOWL

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The purpose of this paper is to describe a simple method of obtaining gastric juice from the ventriculus of the fowl and to report the results of some experiments designed to test the effectiveness of various measures in causing the activation of the glandular stomach or proventriculus.

Methods. A lumbar puncture needle was introduced into the lumen of the ventriculus or gizzard by puncture in the mid-line just below the xiphoid process of the sternum. The animal was placed on its back and held by the assistant, the gizzard was palpated and held in position with one hand while the needle was cautiously passed in with the other. The entry into the cavity of the organ was readily determined by the decrease in the resistance offered to the passage of the needle, as well as by the feeling of contact of the needle with the small stones contained in the gastric mill. The animal did not struggle either during the introduction of the needle, or subsequently to its passage. If the ordinary precautions against infection were taken, the same fowl could be used over and over again. When the needle was in place, the trocar was withdrawn and a Luer or Record syringe attached. The contents of the gizzard could then be aspirated and the effect of the injection of organ extracts or drugs could be followed as desired. The material to be tested was injected either subcutaneously or intramuscularly and the proventricular secretion was aspirated off at intervals, or as it collected. One could in this manner obtain the gastric juice immediately it was produced and any changes in its character could be readily detected.

The effect of sham feeding could be followed in the manner just outlined, an esophageal fistula having been previously produced under ether anesthesia. An animal with an esophageal fistula could be kept

alive for long periods by the introduction of food into the crop through the fistula. Also the effect of mechanical distention of the crop could be followed on such an animal by the introduction into the same of a thin rubber balloon which was afterwards inflated as desired.

The degree of activity of the proventricular glands was determined by noting the rate of secretion and the acidity of the juice in terms of the amount of tenth normal sodium hydroxide required to just neutralize 100 cc. of the juice to Töpfer's reagent. The titration was carried out with fiftieth normal alkali.

The extracts tested out were prepared by boiling the fresh tissue with 0.2 per cent HCl filtering and concentrating the filtrate to a volume about equal to the bulk of tissue used, and neutralizing prior to injection.

Results. It was found that the contents of the gizzard, which were aspirated off immediately after the introduction of the needle, were usually alkaline to Töpfer's reagent. The degree of acidity in those instances where an acid reaction with Töpfer's was obtained was very low. The rate of secretion was very slow unless food or fluid were in the crop. In order to avoid the interfering influence of food or water in the crop, the experiments were carried out for the most part at such times as the crop was empty. The results of several experiments in which injections of extracts were made are shown in tabular form. They indicate that the glands of the proventriculus can be activated to secrete a strongly acid juice by the intramuscular injection of tissue extracts such as those prepared from the proventriculus and duodenum of the fowl, the mammalian gastric mucosa and thyroid gland. Extracts of crop tissue had a slight effect. Extracts of the ventricular lining had no effect, while injection of water was also negative. Pilocarpine had a positive effect.

Sham feeding was very effective in causing a flow of acid juice. In this connection it was found that the effect of allowing water to drip into the throat of the fowl from a pipette was in some instances quite as effective as the voluntary feeding of corn mush. Mechanical distention of the crop had only a very slight effect.

CONCLUSIONS

The fact that it is possible by a very simple procedure to follow the activity of the gastric glands of the fowl makes this animal most serviceable for studies relating to the physiology of gastric secretion. Keeton, Koch and Luckhardt (1) have recently demonstrated the activation of the proventriculus of the duck by gastrin and histamine. They also

found (2) that extracts made from the stomach, duodenum and thyroid glands stimulated secretion in the gastric glands of the dog. The stimulating effect of such extracts upon the glands of the proventriculus of the fowl is therefore consistent with the findings of these authors. The very definite effect of sham feeding and of forced swallowing movements would indicate that the chief factor in the reflex stimulation of the proventriculus was the mechanical stimulation of the pharyngeal mucous membrane.

SUMMARY

A method for obtaining gastric juice from the fowl is described.

The proventricular glands as judged by the acidity and volume of the secretion are definitely stimulated by intramuscular injection of extracts of the proventriculus and duodenum of the fowl, mammalian gastric mucosa and thyroid gland.

The act of swallowing due to forced water drinking in hens with esophageal fistula caused a reflex stimulation of the glands of the proventriculus.

Protocols. Exper. 1. Normal hen. Aspirated fluid alkaline to Töpfer's. Twenty cubic centimeters extract dog's stomach intramuscular.

During next 10 minutes 10 cc. secretion. Acidity 25.

During next 10 minutes 10 cc. secretion. Acidity 50.

During next 10 minutes 10 cc. secretion. Acidity 47

During next 10 minutes 12 cc. secretion. Alkaline due to regurgitation from duodenum.

Exper. 2. Normal hen. One cubic centimeter fluid aspirated alkaline to Töpfers. Twenty cubic centimeters distilled water subcutaneous.

During next 15 minutes 1 cc. juice-alkaline. Fifteen cubic centimeters extract of pyloric mucosa of dog intramuscular.

Three minutes after, juice acid to Töpfer's.

During next 20 minutes 23 cc. secretion. Acidity 35.

During next 20 minutes 21 cc. secretion. Acidity 38.

During next 20 minutes 15 cc. secretion. Acidity 53.

Exper. 3. Normal hen. Six cubic centimeters fluid aspirated. Acidity 18. No further secretion. Eight cubic centimeters extract of crop of hen intramuscular.

During next 16 minutes 5 cc. secretion. Acidity 33.

Fifteen cubic centimeters extract of duodenum of hen intramuscular.

During next 12 minutes 3¼ cc. secretion. Acidity 68.

Ten cubic centimeters extract of proventriculus intramuscular.

During next 12 minutes 4¼ cc. secretion. Acidity 80.

During next 7 minutes 2 cc. secretion. Acidity 70.

Exper. 4. Normal hen. Seven cubic centimeters juice aspirated. Acidity 14.

During next 5 minutes no appreciable secretion.

Ten cubic centimeters extract of proventriculus intramuscular.
Secretion started in 3 minutes.
During next 20 minutes 4 cc. secretion. Acidity 30.

Exper. 5. Normal hen. Ten cubic centimeters juice aspirated. Alkaline to Töpfer's.

Ten cubic centimeters extract of mucosa of dog's stomach intramuscular.
During next 20 minutes 12 cc. secretion. Acidity 39.
During next 18 minutes 6½ cc. secretion. Acidity 36.

Exper. 6. Normal hen. Seven cubic centimeters juice aspirated. Alkaline to Töpfer's.

Fifteen cubic centimeters extract of thyroid gland intramuscular.
During next 30 minutes 10 cc. secretion. Acidity 49.

Exper. 7. Normal hen. Six cubic centimeters juice aspirated. Alkaline to Töpfer's.

Ten cubic centimeters extract of pyloric mucosa of dog.
During next 20 minutes 6 cc. secretion. Acidity 27.

Exper. 8. Hen—esophageal fistula. Four cubic centimeters juice aspirated, alkaline to Töpfer's.

Ten cubic centimeters extract of proventriculus intramuscular.
During next 25 minutes 3 cc. secretion. Acidity 70.
Sham fed after secretion ceased.
During 20 minutes 2.5 cc. secretion. Acidity 40.

Exper. 9. Normal hen Four and five-tenths cubic centimeters juice aspirated, acidity 14.

No secretion during next 10 minutes.
Ten cubic centimeters extract of mucosa of rabbit's stomach intramuscular.
Secretion started in 5 minutes.
During next 5 minutes 5 cc. secretion. Acidity 24.

Exper. 10. Normal hen. Six cubic centimeters juice aspirated, alkaline to Töpfer's.

Ten cubic centimeters extract of crop intramuscular.
During next 10 minutes 1 cc. secretion. Faintly acid.
Ten cubic centimeters extract of gastric mucosa of dog.
During next 10 minutes 7 cc. secretion. Acidity 25.

Exper. 11. Normal hen. Aspirated juice alkaline to Töpfer's.

Ten cubic centimeters extract of lining of ventriculus.
No stimulation of secretion. Few drops aspirated, but reaction alkaline to Töpfer.

Exper. 12. Hen—esophageal fistula. One-half cubic centimeters secretion aspirated alkaline to Töpfer's.

Twenty cubic centimeters water passed into throat from pipette during 10 minutes.
During this period 4 cubic centimeters secretion. Acidity 17.8.
During next 20 minutes 1¾ cc. cubic centimeters secretion. Acidity 28.

BIBLIOGRAPHY

(1) Keeton, Koch and Luckhardt: This Journal, 1920, li, 454.
(2) Luckhardt, Keeton, Koch and LeMer: This Journal, 1920, l, 527.

PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY

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The effect of thyroidectomy on heat production following injury to the suprarenal cortex in rabbits. DAVID MARINE and EMIL J. BAUMANN.

The suprarenal glands have been partially or completely removed or frozen in 42 rabbits. Studies on the respiratory exchange in these rabbits have shown that striking alterations in heat production and CO_2 output may be brought about. These alterations have been grouped under three headings: *a*, Rabbits that live indefinitely after removal or injury of the suprarenals with no appreciable alterations in the respiratory exchange; *b*, Rabbits that show an increase in the respiratory exchange followed by a fall to or below normal, whether dying within 2 or 3 weeks or living indefinitely; *c*, Rabbits that show a fall in heat production beginning within 48 hours after removal or injury of the glands and continuing to death.

These disturbances in heat production appear definitely related to the completeness of the removal of suprarenal function and the duration of life following. Of 15 rabbits with one suprarenal removed and one frozen, or with both frozen, 6 or 40 per cent showed an increase of over 10 per cent in heat production. Of 22 rabbits with complete removal of both main glands 17 or 77 per cent showed an increased heat production. Of 5 rabbits with incomplete suprarenalectomy 1 or 20 per cent showed an increase in heat production. The increased heat production has varied from 10 per cent to 65 per cent above the normal of the particular rabbit. It usually begins within 4 days after removing or freezing the second suprarenal and may continue for 1, 2, 3 or more weeks with or without remissions. Infection, trauma or injury to nerves in removing or freezing may be eliminated as essential causes for the increased heat production.

To obtain data on the question whether the thyroid gland was an important factor in the increased heat production following the partial destruction of the suprarenal, the following procedures have been carried out on six rabbits. *1*, after obtaining control heat production measurements for two weeks *2*, the thyroid glands were removed as completely as possible, and *3*, metabolic studies continued for the next 23 days, when *4*, the suprarenals were removed, and *5*, heat production measurements continued on alternate days until the death or recovery of the animals. A marked drop in heat production following thyroidectomy was obtained in five. In no instance did a rise even above the

myxedema level occur during the first 3 weeks following suprarenalectomy. Two have survived the operations indefinitely; in these the heat production began to rise toward normal during the 4th week. We believe these experiments show that the thyroid gland is an important factor in the increased heat production which follows suprarenalectomy with intact thyroids. Further, it seems probable that the increased heat production is directly or indirectly dependent upon the withdrawal of a regulatory or inhibitory influence normally exercised by the suprarenal cortex. The thyroid and suprarenal cortex are antagonistic while the thyroid and chromaphil tissue, as the work of previous observers has indicated, mutually stimulate each other.

Metabolism studies with enemata of dextrose and levulose. THORNE M. CARPENTER.

The absorption of 6 per cent solutions of Kahlbaum's purified grape sugar was studied after rectal injection in 3 human subjects. The unabsorbed sugar was determined by the Benedict method in enemas taken after the sugar solution had been retained some time. In all cases two enemas were used. With 30 grams of grape sugar in 10 experiments the apparent absorption varied from 17.5 to 27 grams. The time of retention varied from 2 hours and 17 minutes to 6 hours and 24 minutes.

The absorption of Kahlbaum's commercial levulose when introduced rectally was determined in the same manner, using 5 and 10 per cent solutions. In 4 experiments with 25 grams the absorption varied from 16.2 to 21.8 grams and the time of retention was approximately 3½ hours. With 50 grams in 5 and 10 per cent solutions the absorption varied from 24.8 to 48 grams, the duration of retention varying from 1 hour and 29 minutes to 4 hours and 35 minutes.

Levulose was apparently better absorbed than grape sugar when the comparison is made on the basis of per hour values.

The gaseous exchange was determined at the same time that the absorption experiments were made. For this purpose, a half-face mask, Thiry-Tissot valves and two 100-liter Tissot gasometers were used. The expired air was collected in continuously succeeding periods of 5 to 10 minutes duration and samples collected over mercury were analyzed by means of the portable type of the Haldane gas analysis apparatus. Usually the measurements were made for about an hour before the injection began and continued for from 2 to 6 hours.

Control experiments in which the respiratory exchange was measured were carried out using 0.6 per cent sodium chloride solution. There were no marked changes in the respiratory quotient except when the subjects fell asleep, when there was a decided lowering of this ratio, amounting to from 0.05 to 0.07. This drop was usually accompanied by similar parallel changes in the pulse rate.

After rectal injection of 6 per cent grape sugar solution the respiratory quotient increased in 5 out of 7 experiments from 0.03 to 0.10 in from 1 to 3 hours after injection began. Two experiments with 500 cc. of 5 per cent alcohol and 30 grams of grape sugar gave negative results.

In none of the experiments was there a significant change in the oxygen absorption which can be ascribed to the rectal injection. In only one was there a definite increase in pulse rate, (5 beats per minute), which occurred about 3 hours after injection began.

In 4 experiments with 25 grams of levulose in 500 cc. of 0.6 per cent sodium chloride solution there was no definite change in the respiratory quotient or oxygen absorption while in two of them there was an increase in the pulse rate of 5 beats per minute about 1 hour after injection began. In 2 experiments with 50 grams in 500 cc. there was an increase of 0.03 to 0.05 in the respiratory quotient after 1 to 2 hours. In one out of three experiments with 50 grams of levulose in 1000 cc. there was an increase of 0.15 in the respiratory quotient within 3 hours after the injection began, but no definite increase in the oxygen absorption or pulse rate.

These experiments with enemata of dextrose and levulose indicate that 20 grams are absorbed in 3 hours and may raise the respiratory quotient, thus showing that they are utilized in the metabolism.

Reasons for believing that respiratory X is not C_H. YANDELL HENDERSON.

Low oxygen stimulates respiration. So also does CO_2, but through the C_H of the blood. Following the principle of economy of causes the most investigators have held that low oxygen must also alter the C_H of the blood or of the cells of the respiratory center itself. Against this view is the fact that low oxygen induces not acidosis but alkalosis; also the fact that CO_2 stimulates respiration even after vagus section, but low oxygen apparently does not. Under morphine the responsiveness to CO_2 is greatly reduced, while low oxygen is still an effective stimulant.

Respiratory X is the hypothetical substance occurring in the blood under low oxygen. It is not an acid. Some evidence suggests that it does not act upon the respiratory center but stimulates the pulmonary vagal endings. It might be a sulphur compound, for H_2S is a powerful respiratory stimulant capable of causing over-breathing followed by fatal apnea. After vagus section sulphide is merely depressant; its stimulant action is therefore like that which respiratory X, by hypothesis, should have upon the pulmonary endings. Sulphide is rapidly oxidized in the blood. It does not act through C_H. Recent and as yet unpublished investigations, indicate that low oxygen must act through a substance of these physiological properties, although apparently respiratory X cannot be simply H_2S.

The following experiments were mentioned: On Pike's Peak merely squeezing a hand bulb or even opening and closing the fist sufficiently rapidly to fatigue the forearm caused, in Y. H. and some others, but not in all persons, marked hyperpnea. At sea level a similar experiment can be performed, but only on very sensitive subjects, by sawing wood, using both arms vigorously but no other muscles. The significant fact is that the hyperpnea thus induced is followed by apnea. Apparently some substance, in addition, to CO_2 is produced in the overworked and thus anoxemic muscles, which passing into the blood causes

over-breathing. It is quite certain that the condition induced in the blood by over-breathing is alkalosis (low ratio of H_2CO_3:$NaHCO_3$) not acidosis. Therefore the substance is not an acid, but a respiratory stimulant of some other type.

Does the partial pressure of oxygen in arterial blood during progressive anoxemia support the secretory theory? C. W. GREENE and C. H. GREENE.

We have studied the passage of oxygen through the pulmonary epithelium into the blood in an effort to secure additional evidence for or against the secretory theory. Haldane's advocacy of the theory of oxygen secretion is based upon data obtained by the indirect carbon monoxide method. We have directly determined the arterial oxygen content in the present study of the problem. Dogs were used as experimental animals, since they are exceptionally resistant to the effects of low oxygen tensions. Animals under lighter chloretone analgesia were made to rebreathe air from which the carbon dioxide was absorbed until the amount of oxygen was reduced to a point below that necessary to maintain respiratory and circulatory activity. Oxygen determinations were made on samples of arterial blood and alveolar air drawn simultaneously. Samples were taken at different levels of oxygen tension, varying from that of normal air to the lowest alveolar oxygen tension capable of supporting respiration. Alveolar air was collected from a deep bronchus, and the sample was analyzed by the Haldane method. The blood gases were determined by a modification of the micro-method of Van Slyke.

Our experiments show that the oxygen tension in the arterial blood is consistently below that of the alveolar air. The percentage saturation of the arterial blood was followed through the whole range of response to anoxemia including the final collapse at an alveolar oxygen tension of 15 mm. If oxygen secretion occurs it should be called forth under these conditions. The degree of saturation of arterial blood when equilibrated in vitro with air of lowered oxygen tension can be calculated from the dissociation curve for dog blood determined by Barcroft and Camis. The experimentally determined percentage saturation of the arterial blood in vivo was less than that theoretically possible when equilibrated in vitro with a sample of alveolar air taken at the same time. Barcroft and collaborators found that human blood absorbed oxygen when equilibrated in vitro with a corresponding sample of alveolar air. We have found the same to be true for dog's blood through the entire range of oxygen tensions studied. Section of the vagi was without effect upon the oxygen content of the arterial blood. No evidence was obtained to indicate an active secretion of oxygen. The experiments all confirm the view that the passage of oxygen through the lungs into the blood is entirely determined by the purely physical process of diffusion.

TABLE 1

Degree of saturation of arterial blood in dogs 9 and 11 breathing air progressively reduced in oxygen tension

DOG	EXPERIMENT	ALVEOLAR AIR			ARTERIAL BLOOD			
		Oxygen	Oxygen tension	Carbon dioxide tension	Oxygen capacity	Oxygen content	Saturation determined directly	Saturation, calculated from Barcroft's dissociation curve
		per cent	*mm.*	*mm.*	*volume per cent*	*volume per cent*	*per cent*	*per cent*
9	31	14.38	100.6	33.8	12.77	12.08	94.6	91.0
	32	8.61	64.0	24.9	12.77	10.47	82.9	84.0
	33	3.75	27.9	19.2	12.77	3.66	28.7	30.0
	34	6.06	45.0	22.9	12.77	7.01	54.9	60.0
	36	18.60	138.2	15.5	12.77	12.30	95.0	
11	47	1.90	14.6	14.8	7.88	0.83	10.5	12.0

Injury, recovery and death. W. J. V. OSTERHOUT.

Measurements of electrical conductivity show that after a short exposure to a toxic solution tissue recovers completely when returned to the normal environment. But after a longer exposure recovery is incomplete. This does not appear to be due to the death of some the cells but rather to a permanent lowering of the resistance of all of the cells. These experiments are confirmed by similar experiments in which rate of respiration is used as a criterion in place of electrical conductivity.

Elective localization of bacteria following various methods of inoculation, and the production of nephritis by devitalization and infection of teeth in dogs. EDWARD C. ROSENOW and JOHN G. MEISSER.

During the course of numerous experiments on the localization of bacteria from various diseases, instances of extremely specific effects were noted. It must not be assumed that intravenous injection is essential for bacteria to localize in the tissues for which they have elective affinity. Characteristic localization has been obtained repeatedly following other methods of inoculation.

Suppurative pulpitis and hemorrhagic edema of the periosteum opposite the roots of teeth in animals followed intraperitoneal injection of the streptococcus from the pulp of a tooth of a patient who had recurring attacks of pulpitis, dental neuritis, and myositis. Localization in the alveolar epithelium of the lungs in guinea pigs occurred after intraperitoneal injection of the highly virulent green-producing streptococcus from pulmonary influenza. Localization of streptococci, with leukocytic infiltration, was found in the ileum following the intratracheal injection of a small amount of hemorrhagic mucus containing streptococci from a patient with intestinal influenza; hemor-

rhagic colitis developed after the freshly isolated streptococcus, in oil suspension, from another patient with intestinal influenza was injected into the stomach.

The extreme specificity for muscle tissue of a streptococcus from a case of myositis was shown in an experiment in which the organism injected intravenously not only localized in the muscles of a pregnant rabbit, but also in the corresponding muscles of the fetuses. In another marked myositis in which the organism was isolated from the tonsils, lesions in muscles were produced not only by injection of the live streptococcus, but also by large doses of the formalin-killed streptococcus. In the latter a striking capillary reaction was a factor in the production of the lesions; marked swelling of the endothelial lining of the capillaries in the muscles surrounding the lesions was noted. A partial tension streptococcus isolated from a tooth of a patient with recurring attacks of iritis, myositis, and arthritis produced iritis, myositis, and arthritis on intravenous injection, and also produced iritis, including the uninjected eye, and lesions in muscles, when extremely small doses were inoculated into the anterior chamber of one eye. These and numerous other experiments indicate that specific localization in distant tissues or organs may occur from chronic localized, often symptomless, infections.

It has been shown that the bacteria are in close proximity to the newly forming blood vessels in chronic foci of infection, such as granulomas at the apices of devitalized teeth, and are not incapsulated in scar tissue. The fundamental requirements of the principles involved in the production of lesions remote from a focus of infection have been fulfilled, but in order to test the effect of one type of chronic focus of infection, produced so frequently in dental practice through the devitalization of teeth, teeth in dogs were devitalized and infected with bacteria having elective affinity for various tissues.

In the experiments on nephritis the teeth of four dogs were devitalized and infected with a staphylococcus from the nose and tonsils of a patient with advanced nephritis. This strain had a marked affinity for the kidneys of rabbits and produced pronounced lesions on intravenous injection. Catheterized specimens of urine from all the dogs were normal at the beginning of the experiment. The urine was examined at intervals during the course of the experiments, which ranged from 63 to 115 days. One kidney was removed from each dog in from 51 to 104 days after the devitalization and infection of the teeth to afford opportunity to compare the findings in the kidneys removed with the findings in the other kidneys at necropsy.

The four dogs developed lesions in the parenchyma of the kidneys. In three the lesions were more pronounced in the kidney which was examined at necropsy; in the other the reverse was true, and healing was well advanced. The lesions in the kidneys were focal in character, often widely disseminated, and in some instances a large part of the parenchyma of the kidney was involved. The location and type of cellular infiltration resembled closely those of acute interstitial nephri-

tis. In one of these dogs marked cystitis and pyelitis, with secondary infection, were found, and in the adherent membranous exudate marked calcareous deposits had occurred.

The staphylococcus was isolated at the end of the experiment from the infected teeth and from the kidneys of the dogs. It manifested elective localizing power for the kidneys of rabbits when injected intravenously. It was demonstrated in the lesions and proved absent in the normal portion of the kidney.

A new factor in drug analgesia. H. G. BARBOUR and D. S. LEWIS.

Since antipyretic drugs have been found to increase the fluid content of the blood in fevers, one must entertain the possibility of a similar effect in other pathologic conditions. Recently (Cushing and others) certain types of headaches have been relieved by the administration of concentrated sugar or salt solutions apparently owing to a dehydrating effect upon the brain. This also points toward the explanation of drug analgesia which we have to offer.

Thus far in five cases of headache we have studied the blood concentration before and after administration of acetyl-salicylic acid. In each case the criterion has been the percentage of blood solids (done in duplicate) supplemented by red blood cell counts and hemoglobin (Palmer) estimations. Three of the headaches were completely relieved by the drug. As nearly as could be diagnosed they were due respectively to frontal sinus inflammation, psychoneurosis and hypersensitivity to protein food. These showed respective decreases in the blood solids percentages of 0.7, 3.0 and 1.3 at the end of 40 minutes. The corresponding red cell counts decreased by 0.9, 0.8 and 0.8 million and the hemoglobin percentages by 3, 8 and 5.5.

The fourth was a case of pre-arranged headache (induced by going without breakfast) and here the drug relief was not accompanied by changes in the blood concentration.

In the fifth case acetyl-salicylic acid gave no relief; correspondingly no change in the blood concentration could be detected. Similarly, the drug given to a normal individual apparently increased the concentration of the blood.

The pharmacologic relief of certain types of headache is evidently associated with dilution of the blood.

The "protein" headache was studied in a fasting condition and control determinations made on four different days with the subject also fasting showed that the hemodilution of drug analgesia is not a hyperdilution but is probably rather a return to normal from a condition of over-concentration.

Integumentary changes in the sheep following thyroidectomy and administration of thyroxin. SUTHERLAND SIMPSON.

Photographs of two rams were shown—the sire a registered Rambouillet ram, and the dams two Shropshire ewes of the same flock. Both were born on the same day, May 7, 1920. From one the thyroid

gland was removed at the age of about 5 months and the other was kept as a control. At the time of operation, the horns were approximately of the same size in each, but subsequently the difference in the rate of growth was most marked. In November 1921 when the rams were about 18 months old, in the thyroidectomized animal the left horn from root to tip measured, along the greater curvature, 6 inches and the right 4 inches. In the control (normal) ram, the corresponding measurement of each horn was 21 inches.

In a thyroidectomized lamb—a cretin dwarf—at the age of 9 months, thyroxin was administered subcutaneously in ½ mgm. doses daily. After about a fortnight of this treatment the wool began to fall out. Within a week the whole fleece had been shed leaving the skin completely denuded except for a few tufts on the head. This happened in the end of February 1921 in the western part of the state of New York when the weather was at its coldest.

The shedding of the wool was possibly caused by the excessive dosage of thyroxin. When thyroxin was reduced a normal fleece developed.

Blood flow and oxygen metabolism of the thyroid gland.[1] F. P. KNOWLTON, M. S. DOOLEY and A. N. CURTISS.

The measurement of the blood flow and gaseous metabolism of the thyroid gland was undertaken with the hope that the method might offer a means of studying the activities of the gland under various conditions. This preliminary report deals with resting conditions in dogs. The animals were anesthetized with morphin and urethane, anesthesia being completed with chloroform as needed.

The blood-flow was measured and the blood samples collected by the method of Barcroft and Brodie. The cannula for collection was usually placed in the external jugular vein; all veins except those returning blood from the thyroid being tied. When the thyroid return was in part through the external jugular the cannula was placed in the subclavian. As valves in the veins prevent back flow of blood it was necessary to pass an oiled cannula down the vein past the valve and into the jugular bulb. After flow and samples were obtained the tube was withdrawn. The use of an anticoagulant was an aid in preventing clotting. Care was taken to avoid injury to the nerve supply of the gland and to protect the gland from temperature changes.

The flow in each case is the average of 5 to 10 measurements, and the gas analyses were done in duplicate. The Van Slyke apparatus was used for the oxygen determinations.

The average of our results is a blood flow of 3.55 cc. per gram per minute and an oxygen consumption of 0.0927 cc. per gram per minute. Our blood-flow average is somewhat lower than the figure of 5.9 cc. given by Tschuewsky who calculated the flow in 5 experiments using a stromuhr in the carotid.

The flow and metabolism appear to be of the same magnitude as the

[1] This research has been aided in part by a grant from the research fund of the American Association for the Advancement of Science.

suprarenal, and somewhat higher than in other resting tissues, with the exception of the brain which seems to have a higher rate of metabolism.

Further experiments are in progress dealing with the influence of nerves, of adrenalin, and with other phases of the gland's activities.

Blood flow and oxygen metabolism of the thyroid

EXPERIMENT NUMBER	WEIGHT	THYROID WEIGHT	THYROID RATIO	THYROID-KIDNEY RATIO	BLOOD PRESSURE	BLOOD FLOW	GM.-MIN. FLOW	OXYGEN PER GM.-MIN.	REMARKS
	kgm.		*per cent*					*cc.*	
1	16.8	3.37	0.020	—		6.66	1.97		Male
2	6.4	2.35	0.036	—	110	2.0	8.51	0.201	Old female fox terrier
3	15.0	1.55	0.014	1-27	108	2.07	1.33	0.059	Young male; slow heart
4	11.92	0.97	0.0082	1-30	126	6.21	6.40	—	Female spaniel
5	19.0	3.25	0.017	1-20	104	5.17	1.65	0.045	Male hound
6	11.8	2.08	0.017	1-16	115	5.30	2.55	—	Male hound
7	11.1	1.28	0.012	1-28	122	2.46	1.93	0.0569	Male
8	8.0	1.15	0.015	1-22	114	1.01	0.88	0.0376	Male
9	11.0	0.72	0.006	1-49	114	3.31	4.73	0.1749	Male
10	17.2	1.1	0.006	1-39	116	2.27	2.06	0.0985	Male
11	16.6	2.11	0.013	1-20	126	11.54	5.44	0.180	Male hound
12	15.0	1.3	0.008	1-36	106	2.4	1.84	0.133	Young male
13	21.9	1.15	0.005	1-54	128	7.89	6.94	0.224	Male
	13.9	1.79	0.013	1-26		5.09	3.55	0.0927	Average

Results on an enlarged thyroid gland nine years after obstructing the veins. C. C. GUTHRIE.

At the time of the operation both lobes of the gland showed marked enlargement—the left being somewhat larger than the right. All the major veins to the left lobe were tied, only a few minor branches being left. Following a period of enlargement lasting but a few days the operated lobe greatly diminished in size. The bitch remained in good health and raised numerous litters of puppies. She was killed in a fight almost 9 years later. At this time the operated lobe was perhaps somewhat subnormal in size and microscopically showed normal appearing areas of thyroid alveoli and colloid. More fibrous tissue was present than normally. The unoperated lobe was considerably larger than at the time of operation. No normal appearing thyroid structure or colloid was observed but the picture was that of hyperplasia of an adenomatous type.

The result harmonizes with observations extending over shorter periods of time previously reported.

The after-effect of prolonged fasting on the basal metabolic rate. MARGARETE KUNDE.

There is a number of well controlled experiments on the effects of prolonged fasting on metabolism, but no one seems to have investigated the possible after effect of fasting on the basal metabolic rate. We have extensive data on the macro- and microscopic changes induced in the various organs by fasting. We have reports of apparent improvement in mental condition in man as a result of fasting. The tonus of the stomach increases during fasting. Extensive experiments on some invertebrate groups by Child and his co-workers seem to point to a process of rejuvenescence of tissue by starvation. In diabetes in man it appears that partial starvation may in some cases increase the capacity of the body to utilize sugar.

Since it is established that the young individual has a higher metabolic rate than the adult, it would seem to follow that if prolonged fasting actually leads to rejuvenescence or extensive renewal of body cells over that which takes place under ordinary conditions of feeding, such rejuvenescence would be evidenced by an increase in the oxidative power of the cells.

In these experiments the basal metabolic rate, based on the oxygen consumption was used as an index of the normal functional capacity of the cells when at rest. For this purpose a Benedict portable respiratory apparatus, with suitable modifications, adapting it to our needs, was used.

Two human subjects and three dogs were used as subjects in these experiments. A simple muzzle for connecting the dogs to the apparatus consisted of a perforated rubber dam tightly stretched over one end of a muzzle that serves the purpose of a face mask in human subjects. The muzzle was connected with the three-way valve of the apparatus. Adequate tests for tightness checked to within one per cent. All computations were based on the amount of oxygen consumed as indicated by graphic records.

The entire time during which data was collected for these experiments is divided into three phases. First the control period; second the period of fasting; third the period after fasting. The control period for the different subjects was from two weeks to several months. The fasting period for each human was fifteen days. The fasting period for each of the dogs was forty days. The period after fasting varies with the different subjects from three weeks to seven months and is still in progress.

Precaution for constant postabsorptive condition was observed. For the dogs the food was the same in quantity and quality during the control and the after period, with this exception—when the dogs had regained their normal weight, the amount of food was slightly diminished. Each dog was fed eighteen hours before its basal metabolic rate was taken. The dogs were trained to lie quietly in a state of perfect rest for forty minutes immediately preceding the determination and to remain so for the time during which they were connected to the

respiratory apparatus. This method has the advantage that error due to muscular movement is entirely eliminated.

The results at this stage of the investigation show an increase in the basal metabolic rate as the after effect of prolonged fasting. This increase persists for an extended period of time, the exact length of which has not yet been fully determined.

The oxygen capacity of bird's blood. THEOPHILE K. KRUSE.

In a comparative study of the oxygen capacity of the blood of seven ducks and eight chickens, it was found that the duck has an average capacity of 3 cc. per 100 cc. or 15 per cent more than chicken. In collaboration with Professor Guthrie it was observed that the average blood volume also was the greater in the duck. It is therefore evident that the oxygen blood capacity of the duck is greater. Incidentally it was observed that the weight of heart and lungs relative to body weight was also geater for the duck.

The Van Slyke method for blood analysis was found unsuitable because the reagents recommended form a sticky coagulum, which retains small bubbles of gas and occupies volume in the calibrated portion of the apparatus. It was found that by using a smaller quantity of potassium ferricyanide, coagulum formation, except for slight clot formed by the ammonia solution, was eliminated. Control tests on mammalian blood showed that such procedure is desirable as was shown by Haldane in 1920. The best results were obtained by substituting water for ammonia and one drop of 25 per cent potassium ferricyanide for 0.4 cc. of the saturated solution. Even the bird's blood remains fluid and the gases are extracted with the same ease as when mammalian blood is used. Some carbon dioxide is extracted and is absorbed by 0.5 cc. of 10 per cent sodium hydroxide solution. The oxygen is absorbed by 1 cc. of alkaline pyrogallate solution.

After the completion of the details of this technique the recent publication by Van Slyke and Stadie appeared recommending a similar technique for mammalian blood.

A comparison of the respiratory and circulatory effects of anoxemia and carbon dioxide. EDWARD C. SCHNEIDER.

Low oxygen tension effects were produced by rebreathing 52 liters of air from which the carbon dioxide was removed. The carbon dioxide effects were produced by rebreathing air in which the oxygen content was maintained at 25 or 30 per cent while the carbon dioxide gradually increased. Each variety of experiment was continued about 25 minutes.

In anoxemia the respiratory response gave an average increase in the per minute volume of breathing of 5.6 liters, and of 330 cc. in the depth of breathing; while the rate of breathing was slightly decreased. The effects of a gradual increase in CO_2 were an average increase of 40.4 liters in the per minute volume, of 2 liters in the volume per breath and a doubling of the rate of breathing.

The average circulatory changes of anoxemia were an acceleration

of 28.7 beats in the pulse rate, a rise of 15.8 mm. Hg. in the systolic arterial pressure, a fall of 6.9 mm. in the diastolic pressure, an increase of 22.9 mm. in pulse pressure, no change in capillary pressure, a fall of 4.3 cm. H_2O in the venous pressure, a gradual decrease in the hand volume, and a decrease in the rate of blood flow through the hand when the oxygen in the respired air was less than 13 per cent.

The average circulatory changes under increasing amounts of carbon dioxide were an acceleration of 12 beats in pulse rate, a rise of 21.6 mm. Hg. in the systolic pressure, a rise of 12.1 mm. in diastolic pressure, a rise of 10 mm. in pulse pressure, a rise of 13 mm. Hg. in capillary pressure, a rise of 4.7 cm. H_2O in venous pressure, and an increase in hand volume up to 3.5 per cent CO_2 after which there was a gradual decrease. The blood flow through the hand showed no change until the CO_2 had reached 3 per cent; however, at higher percentages the flow was lessened.

A study of blood platelets. THEOPHILE K. KRUSE.

Blood platelets may be demonstrated with great ease for microscopic observation by mixing the classic preservatives such as citrates, oxalates and fluorides with colloids such as acacia, gelatin and soluble starch. The colloids collect the corpuscles by agglutination and thereby make the platelets visible. The use of these colloids without the anticoagulants permits observation longer than the saline alone, but not over 25 minutes. Mixture with the anticoagulants, however, permits observation for hours. The concentrations of colloids which have been found satisfactory are gelatin 3 to 6 per cent, acacia 5 to 20 per cent and soluble starch 2 to 6 per cent. A half per cent of agar-agar may be used but is not very suitable on account of its jellying property. The concentrations of the anticoagulants which have been found satisfactory are, sodium fluoride 1 per cent, potassium oxalate 2 per cent and sodium citrate 2 per cent. In view of the fact that all of these colloids contain calcium, mixture with the oxalate is not very suitable on account of its precipitation. Sodium fluoride mixed with acacia forms a flocculent precipitate which settles readily and does not seem to interfere with subsequent observation. A mixture of 2 to 4 per cent of soluble starch with 1 per cent of sodium fluoride is probably the most satisfactory colloid mixture. None of these reagents are superior to 2 per cent sodium metaphosphate in 0.9 per cent sodium chloride. This reagent agglutinates the corpuscles and preserves the platelets as well. Sodium metaphosphate is most readily prepared by heating sodium ammonium phosphate in a crucible and pouring the moulten mass on a porcelain or iron plate.

The following method of mounting was found most suitable. Place a drop of reagent on a slide. By finger puncture obtain a drop of blood slightly larger than the head of a pin and bring it in contact with the reagent on the slide. By means of a needle stir the fluid on the slide to insure uniform mixture and enlarge the drop to a size slightly less than that of a cover slip, cover and examine.

Effects of carbon dioxide on protoplasmic viscosity. M. H. JACOBS.

If a protozoan such as *Paramoecium* or *Colpidium* be allowed to ingest India ink and then be subjected to the action of centrifugal force, the ease with which the food-vacuoles can be separated from the rest of the protoplasm may be used as a criterion of the physical state of the latter. The eggs of the sea-urchin, *Arbacia*, may be employed in a similar fashion without any preliminary treatment.

Using this method, it can be shown that a short exposure to carbon dioxide causes a great decrease in protoplasmic viscosity; a longer one an increase. Both effects are, within limits, completely reversible, though the solidification, if too long continued, tends to pass over into an irreversible coagulation. A reversible solidification is particularly easy to demonstrate in the eggs of *Arbacia*. It can also be shown that CO_2-saturated water, applied with a fine capillary pipette, produces an apparent local liquefaction of the protoplasm in *Amoeba* leading to the formation of a pseudopodium. An excess of the solution, after diffusion has occurred, brings about what appears to be a temporary solidification of the entire cell. It is possible that local alterations in the rate of CO_2 production may be one of the factors concerned in amoeboid movement.

The effects in question are not due to a lack of oxygen and are probably closely connected with an increased acidity of the protoplasm, since in suitable material (eggs of the starfish stained with neutral red) it is easy to demonstrate an increase in intracellular acidity within a few seconds after exposure to the concentrations of CO_2 employed. The degree of acidity produced does not depend on the pH of the external medium as such but rather on its CO_2 content. Sea water brought to a pH of 6.0 by the addition of carbon dioxide produces intracellular acidity in the eggs of the starfish and solidification in those of *Arbacia*, while the same pH brought about by the addition of HCl and removal of the CO_2 set free from the bicarbonates has neither of these effects.

It is suggested that in the mammalian body a CO_2 deficiency completely compensated by a proportionate decrease in bicarbonate and a CO_2 excess completely compensated by a proportionate increase in bicarbonate, while giving the same pH as measured with the hydrogen electrode or an appropriate indicator, should in reality have different physiological effects on the living cells of the body.

Water intoxication. LEONARD G. ROWNTREE.

Reference was made to the peculiar form of intoxication noted by Larson, Weir and Rowntree which resulted from excessive intake of water subsequent to the subcutaneous use of pituitary extract. They observed this intoxication in cases of diabetes insipidus and subsequently in normal dogs. Three patients with diabetes insipidus, subsequent to receiving subcutaneous injections of pituitary extract, continued to take water in the amounts to which they had become accustomed. Within a few hours headache, nausea, asthenia, incoördination and staggering gait developed. The reaction was extremely severe and

occasioned great concern. In dogs, water intoxication was produced readily after the administration of pituitary extract. The symptoms were asthenia, restlessness, frequency of urination, diarrhea, nausea, retching, vomiting, salivation, tremor, muscle twitching, ataxia, convulsions, frothing at the mouth, stupor and coma. Intoxication ended often in death. This condition could not be obtained by pituitary extract or water alone but only by the combination of the two.

Considering it likely that water was responsible for the production of these symptoms and that pituitary extract favored their development through lessening the amount of water excreted by the organism, the work was continued by Rowntree. In subsequent experiments, 50 cc. of water per kilo of body weight every half-hour instead of every hour, resulted regularly in the appearance of this same train of symptoms. Tap water and distilled water proved equally toxic and the symptoms developed irrespective of the temperature of the water.

Water intoxication was produced in dogs, cats, rabbits and guinea pigs. The influence of previous fasting, previous water deprivation and water excess was determined. Intoxication could not be produced by the intravenous administration of salt solution, in fact, animals in the early stage of water toxicity withstood intravenous injections of large quantities of normal salt solution. One crucial experiment carried out with the assistance of Doctor Meek showed an increase of intracranial pressure equivalent to 35 centimeters of water. Subsequently, in animals already in water intoxication, attempts to decrease intracranial pressure by the use of hypertonic salt solution intravenously prevented convulsions and in some instances caused almost immediate disappearance of symptoms of toxicity. In one dog receiving 90 cc. of 10 per cent saline, paralysis of the hind legs developed. Paraplegia was also encountered in the second experiment.

The significance of water intoxication is not yet clear. However, it is of interest that such a train of toxic manifestations from the excessive ingestion of water by mouth results. In uremia, the same train of symptoms is noted clinically. The frequent association of uremia with wet brain and the marked temporary improvement in uremia following sweating are well known clinically. It is possible that water intoxication plays some rôle in uremia.

Blood volume changes following water abstinence. NORMAN M. KEITH.

Several investigators have studied the changes in a unit volume of blood in dogs not taking food or water. Divergent results have often been obtained even under similar experimental conditions. My experiments were carried out for the purpose of ascertaining whether there were any definite changes in the total blood volume which might explain the widely different results when only a unit volume of blood was considered. Groll, in 1887, demonstrated that there was a distinct rise in the hemoglobin content of venous blood in a dog not taking food and water for 21 days. He also demonstrated that when fluid was administered there was a rapid decrease in hemoglobin percentage,

with marked improvement in the general condition. In a similar observation which extended for 28 days I found that the administration of fluid and food resulted in a 36 per cent decrease in hemoglobin, within 24 hours. Two months later in a similar observation on the same dog, these remarkable changes in the hemoglobin content were associated with a striking decrease in the volume of circulating blood, particularly the plasma fraction. In six further experiments there was always found to be a marked shrinkage of the circulating blood and plasma associated with the continued abstinence from water. The hemoglobin percentage was usually though not invariably increased. The whole blood viscosity was always increased. In one animal with a very low blood volume and high viscosity the blood pressure was found to be reduced; the tracing showed a pressure between 70 and 75 mm. of mercury. Studies were made on serum and plasma in order to determine whether the reduction in total plasma was accompanied by an increase in viscosity and in protein and chlorid concentrations. The results obtained were not uniform. In three experiments there was an increase in the plasma viscosity and also in the protein and chlorid concentrations. In two other experiments there was no increase either in viscosity or protein concentration, although the chlorids were increased. Thus there appeared to be two distinct reactions in the blood serum, *a*, an actual concentration; and *b*, some equalizing factor in the tissues which prevented definite protein concentration. Consideration of this difference in the reaction of the serum needs further elucidation which it is hoped will be possible by further experiments. If food and water is administered to these animals after a considerable period of fasting, they almost invariably recover their original activity rapidly. The accompanying changes in the blood are striking and include a rapid increase in the amount of circulating plasma, a fall in hemoglobin, and a decrease in the viscosity of the whole blood, plasma and serum.

The demonstration of a decrease in the volume of the circulating blood, in this condition offers an explanation for similar clinical conditions, such as chronic shock-like states and atrophic infants. It also affords an experimental method for the study of rational therapy in such cases.

Smooth muscle responses when subjected to alcohols. F. M. Baldwin and B. M. Harrison.

A comparative study is made on responses of excised muscles of the esophagus, stomach, intestine and oviduct of the frog and turtle and body muscles of the earthworm when immersed in ethyl and propyl alcohols ranging in strength from 40 to 1.56 vol. per cent, and from 10 to 0.5 vol. per cent, respectively. Ethyl alcohol on frog tissues in concentrations above 12.5 vols. per cent is markedly inhibitory and responses are accompanied by loss of irritability. Concentrations which produce most marked sensitization range between 3.12 vols. per cent and 6.25 vols. per cent. Concentrations below this result in a gradual diminution of response, although somewhat augmentory down as low

as 0.25 vol. per cent. The same alcohol on earthworm muscles effects its optimum sensitization in concentrations ranging from 1.2 vols. per cent to 2 vols. per cent and augmentation is about three times the normal. Below 0.5 vol. per cent little difference in responses from those of normal is noted. Propyl alcohol on earthworm muscles is inhibitory in concentrations above 5 vols. per cent, the most marked sensitization being in the neighborhood of 1 vol. per cent. Tissues in higher concentrations of both alcohols fatigue quickly and maintain contracture indefinitely. Weak solutions below the optimum enhance irritability and fatigue processes are delayed.

The facts compare favorably with previous work on voluntary muscles and substantiate a belief that their physiological condition may be markedly altered by changing the physical state of their environment.

Thermal effects accompanying alteration of the O_2 and CO_2 content of blood. HOWARD W. HAGGARD.

Experiments reported demonstrate that the reaction of reduced whole blood with O_2 or CO is practically athermic. The combination of laked blood or hemoglobin solutions with either gas is exathermic. The amount of heat actually liberated is influenced by the state of aggregation of the hemoglobin. Results to be reported suggest the probability that there is an endothermic absorption which neutralizes the liberation of heat by the combination of hemoglobin with O_2 or CO. The shift of the equilibrium $HbO_2+CO \rightleftarrows HbCO+O_2$ under variation of temperature depends upon the relative heating effects of the combination of the iron of hemoglobin with O_2 and CO. Absorption of CO_2 by the blood is attended with the evolution of heat, and the loss of CO_2 by the absorption of heat.

Some variations noted in the ash of the sweat. G. A. TALBERT.

Camerer seems to stand quite alone on the determination of the ash of human sweat and he confined himself to the sweat produced from heat.

In my investigations I found that the ash of work sweat is quite perceptibly higher than that of heat sweat, which is in harmony with determinations of Pugliesse on the horse.

There is quite a definite regional variation to be observed; the chest yielding the higher ash, a little less from the arm and always the least from the leg. The covered parts of the body always yield a higher ash than the corresponding uncovered. There is some evidence that this might be a function of skin temperature. The parts that yield the higher acidity yield the smaller ash.

A radial transmission sphygmograph with rigid support. CARL J. WIGGERS and WILLIAM R. BAKER (by invitation).

The fundamental principles to be applied in the construction of sphygmographs have been carefully analyzed by Frank and Petter.[1] In no existing instrument, however, has the question of a rigid support

[1] Frank and Petter: Zeitschr. f. Biol., 1907, xlix, 70.

been worked out satisfactorily. This is due to the fact that the basal frame work is really designed to fit upon a flat surface rather than over the outer edge of the radius, as it should be, to allow the adjustment of the button to the radial artery itself. Consequently, when a top heavy wrist-sphygmograph with such a support is strapped on, it tends to swing inward and outward, see-sawing on its base over the outer edge of the radius.

To obviate this difficulty of fixation and retain all the advantages of a construction based on the Frank-Petter principles, we devised a new form of transmission-sphygmograph which, when used in connection with optical recording capsules, reproduces unvarying pulse contours at different spring tensions and is therefore adequate for registering the contour of the peripheral pulse with accuracy. It may also be used with polygraphs of various designs but it should be emphatically stated that the accuracy of the reproduced pulses then depends on the quality of the particular recording tambour.

The details of the apparatus are shown in figure 1. Fixation is obtained by fastening to the lower forearm a median plate fitted to conform to the contour of the arm. The sphygmographic portion consists of a button, *a*, an L-shaped lever, *b*, pivoting on steel points, a spring, *c*, for adjusting its tension by a convenient knob, *d*, and a receiving tambour, *e*, in contact with the upright limb of the L-shaped lever. All of this portion is rigidly mounted and can be rotated on an upright rod, *f*, fastened to the median plate. Used on the left arm, the button and vertical lever are swung to the left; used on the right arm, they are swung to the right as shown in figure 1. This swing has the further advantage that the radial artery may be palpated during the process of adjustment and assurance obtained that the point of greatest pulsation is actually recorded.

Fig. 1

The technic of its application and reproduced records obtained from normal and clinical cases were shown. The following dimensions of parts may be added: Median supporting plate length = 9 cm.; width, 5 cm. above tapering to 4 cm. distally. Vertical arm of lever 5.5 cm., horizontal arm, 1 cm.; magnification, 5 ×. Variation of spring tension-counterbalanced by from 50 to 250 grams placed on button. Diameter of receiving tambour 4.5 cm.—diameter of plate 2.3 cm.

A model demonstrating the dynamics of mitral regurgitation. CARL J. WIGGERS and HAROLD FEIL.

In mitral insufficiency experiments we observed that as long as the intraventricular pressure rose rapidly the regurgitation into the auricle was very slight during the isometric contraction phase. A physical model was demonstrated to show that this is not due to any compensatory phenomenon but to the physical fact that *during the isometric contraction phase time is not available to overcome the inertia of the fluid and set it in motion.*

Physiological aspects of experiments on mitral regurgitation. HAROLD FEIL and CARL J. WIGGERS.

Mitral regurgitation was produced by the method described by one of us[1] which not only has the advantage that immediate as well as permanent reactions can be studied but is of additional value because normal and pathological conditions can be alternated repeatedly during the course of any experiment and the results thus controlled.

An analysis of pressure curves from various cardiac chambers and large vessels showed the following results during incidence of a lesion.

Left Auricular Pressure: No abnormal elevation of pressure during the isometric contraction phase; marked increase during systolic ejection and early diastolic inflow phases.

Left Ventricular Pressure: Slight elevation of initial tension; very slight or no decrease in gradient of isometric pressure rise; no change in isometric interval; lower pressure maximum during systolic ejection. These observances together with volume curves of left auricle showed that there is only an insignificant or no regurgitation during the isometric contraction phase but that the chief regurgitation occurs during systolic ejection and extends 0.08–0.09 of a second into diastole.

Pulmonary Artery. No elevation of systolic or diastolic pressure indicating *a*, that output of right ventricle is unchanged; and *b*, that no "back pressure effect" occurs as long as heart muscle is in good condition.

Since the left auricular pressure maximum failed to rise after 3 or 4 beats, since aortic pressures gradually rose and pulse pressures returned to normal, the only conclusion could be arrived at that left and right ventricles were ejecting equal volumes within a few beats after production of a lesion. Since no evidence of any altered output of the right ventricle was obtained the only possible conclusion was that the systolic discharge of the left ventricle is restored to normal during a mitral leak. This is accomplished by a larger tidal volume entering the ventricle and the increased stroke resulting from the elevation of initial tension.

Physiological interest in these experiments centers chiefly in the following facts:—

1. An isometric mode of contraction during early systole is possible even when no valves exist between auricle and ventricle for in such cases no significant regurgitation takes place until the aortic valves have opened.

2. The mitral valves are not necessary to maintain normal and equal systolic discharges from right and left ventricles, the latter compensating within 3 or 4 beats by moving a larger tidal volume during each systole.

3. The increased auricular volume resulting from regurgitation during the first few beats is accommodated in the left auricle and tributary veins and does not act to elevate pressures in the pulmonary circuit and right heart.

[1] Wiggers: Arch. Int. Med., 1915, xvi, 135.

The contour of the pressure variations in the portal vein. DONALD D. FORWARD and HAROLD FEIL.

By inserting the narrow cannula of an optical manometer into the main portal trunk of the portal vein via the gastro-splenic branch, the contour of the portal pulse was studied under a variety of conditions and time relations with the heart sounds and aortic pulse were established.

The results and interpretations may be summarized as follows:

1. A portal pulse consisting of two waves is produced in the portal vein during each cardiac cycle.

2. The first wave "b," we interpret as due to extraneous impact and traction, the second or real wave "d" we believe due to the changing balance which occurs between inflow and outflow of the portal system, at different times of the cardiac cycle.

3. Our interpretation of the d-wave is as follows:- Following each systolic ejection a certain pulse volume reaches the splanchnic vessels. Owing to the splanchnic resistance there is a delay of 0.02—0.07 of a second (average 0.04 of a second) in the transferance of this increased volume to the portal vein. Consequently, the portal pressure begins to rise later than the aortic pulse by this interval. This transfer of blood does not cease with the end of systole but continues into diastole, consequently, portal pressure reaches its maximum relatively early in diastole. At this stage, the splanchnic inflow exactly equals hepatic outflow and wave "d" has reached its summit. Thereafter outflow exceeds inflow and portal pressure declines.

4. The amplitude of the chief or "d" wave is determined by *a*, the length of diastole; *b*, by the pulse volume entering the portal vein. The latter is determined by *1*, the systolic discharge of the left ventricle; and *2*, the degree of splanchnic constriction or dilatation.

5. Evidence to this interpretation of the b and c waves, the b-c interval represents the time required for the systolic pulse volume to be transferred from the splanchnic tributaries to the portal vein. This interval is unaffected by changes in heart rate or moderate saline infusion but is increased whenever the splanchnic vessels are constricted, and decreased whenever they are dilated.

On the specificity of gastrin and pancreatic secretin. ARNO B. LUCKHARDT, S. C. HENN and W. L. PALMER.

The presence of gastrin (or gastrin bodies) in the duodenal mucosa, liver, and thyroid glands tacitly questioned the physiological significance of this substance previously described as present in the gastric mucosa. Since its mode of preparation from duodenal mucosa is not much different from the method of preparation of pancreatic secretin by Bayliss and Starling, also obtained from duodenal mucosa, it seemed desirable to determine whether gastrin stimulated not only the stomach but also the pancreas and to investigate whether a pancreatic secretin could be prepared from the gastric mucosa as well as intestinal mucosa which on injection would stimulate both the pancreas and the stomach to secretory activity.

In dogs, whose stomachs, provided with gastrostomies, were ligated off at the cardia and pylorus, and who in addition possessed pancreatic fistulae and jejunostomies, the deep muscular injection of gastrin prepared from gastric or intestinal mucosa induced a pronounced secretion of gastric juice and a marked secretion of pancreatic juice. In such animals pancreatic secretin preparations however made stimulated not only the pancreas but greatly increased the volume and the acidities of the gastric juice. Preparations of pancreatic secretin obtained from the gastric mucosa were as active in stimulating the pancreas to activity as were secretin preparations made from the intestinal mucosa. In such animals HCl acid introduced into the duodenum or jejunum caused a secretion only of pancreatic juice, whereas pancreatic secretin preparations stimulated both the stomach and the pancreas.

There is, therefore, no specificity of the so-called gastrins and secretins. Gastrin can be obtained furthermore from the duodenal mucosa; pancreatic secretin can be obtained from the gastric mucosa. Since the introduction of HCl acid into the bowel caused only a secretion of pancreatic juice it is concluded that the secretin absorbed from the gut under the influence of HCl acid placed therein is a different substance than is present in HCl acid extracts made from gastric or intestinal mucosa.

The penetration of dyes into living cells. MARIAN IRWIN.

The penetration of dyes into the living cells of *Nitella* has been quantitatively investigated. Quantitative studies have not hitherto been possible because the amount of penetration could not be determined with sufficient accuracy. The cell of *Nitella* is so large that the sap can be squeezed out of a single cell and examined colorimetrically.

The electrical conductivity of animal tissues under normal and pathological conditions. GEORGE W. CRILE, HELEN R. HOSMER and AMY F. ROWLAND.

The researches of Lillie, Loeb, Osterhout, Mathews, McClendon, Hill, Lucas and of many others indicate that the functions of the cells of living organisms are related to electrical processes; that the living cell, whether it exists alone or as an element in a complex organism, possesses a certain store of potential energy which is manifested by variations in polarity and by action currents; that variations in the permeability of the living cell to the electrically charged elements of the fluid which surrounds it parallel variations in irritability in response to stimulation; that factors which suspend or abolish irritability also suspend or abolish alterations in permeability.

The facts of bio-electric currents indicate the importance of further investigation. For example:—

What is the range of the electric conductance of living tissue? How does that range compare with that of other electrical conductors? Is the range of conductance the same for all types of tissue, and in each tissue does it remain constant? Is the electric conductance of each

tissue a factor in the production of the activities of the organism, to which a fairly constant value can be assigned?

In an attempt to answer these questions the electrical conductivity of 4764 sections from 455 rabbits, and 219 sections of pathological human tissues have been measured. From the findings the following general conclusions are drawn:

1. Influences which affect the general physical condition of the organism produce changes in electric conductivity in the dominating reactive tissues, these changes being uniformly and measurably manifested in the brain and the liver. Apparently these changes in conductivity appear more promptly than any gross clinical alteration.

2. Apparently the liver is more promptly and more markedly affected than any other tissue, as animals showing either no or very slight changes in the cerebrum and cerebellum will often show a marked alteration in the conductivity of the liver. On account of the wide variation in liver measurements, the effects of applied agents are best determined by measurements of the cerebrum and cerebellum.

3. A study of the individual measurements seems to indicate that the variations represent slightly different stages in a process that varies in rate in different animals and in the different organs of the same animal.

4. In view of the evidence we feel justified in the assumption that the first effect of stimulation within the organism is a slight decrease of the conductivity of the liver followed by a rapid continuous rise to above the normal as the state of exhaustion approaches; there is a slight and prompt increase in the conductivity of the cerebellum followed by a gradual continuous fall, and also a relatively slower increase in the conductivity of the cerebrum followed a gradual continuous decrease.

5. These studies indicate that electric conductivity measurements provide a means of interpretation of the normal operation of the organism, and whereby to measure the progress of certain pathological processes with the various organs and tissues.

6. From our findings to date, it would appear that the intracellular changes in exhaustion and shock which are revealed by the microscope are paralleled by alterations in electric conductivity, and that both the histologic and the electric changes bear a direct relation to the vitality of the organ.

Ammonia, a causative factor in meat poisoning in Eck-fistula dogs. S. A. MATTHEWS.

I. Physiological rôle of the portal circulation.

a. Apparently unimportant. Animals (dogs) with Eck-fistula enjoy perfect health on a liberal diet of table scraps—(meat, bones, bread, etc.).

b. As a rule Eck-fistula dogs do not maintain good health on a heavy meat diet, and about 25 per cent will not tolerate any exclusive meat diet. In from two to three days of heavy meat feeding, a train of nervous symptoms develops, resembling tetany, which passes on to

depression and death. From the first the stomach ceases to empty itself properly and when the symptoms are well developed (generally about the third day of feeding) nearly all of the meat previously fed can be recovered from the stomach.

There is little tendency to vomit during the sickness. If the stomach be emptied by induced vomiting or by lavage, recovery sets in promptly and as a rule is complete in from six to eight hours. If the animal is in coma, the injection of glucose (25 gm.) will hasten recovery.

As meat poisoning cannot be induced in normal dogs, a protective function was attributed to the liver, by Hahn, Massen, Nencki and Pavlov (1893). Our observations suggest a like conclusion.

II. Nature of the poison.

a. Normal blood (general circulation) contains no appreciable NH_3. The blood of the portal vein may contain as much as 0.1 to 0.2 mgm. per 100 cc. During the development of meat poisoning, the NH_3 in the blood of the general circulation reaches 1.8 to 2.2 mgm. per 100 cc. Ammonia above 1 mg. per 100 cc. is always accompanied with nervous symptoms often severe enough to produce coma. The heart is slow and irregular and the iris is contracted to a pin-point. Both conditions are relieved by atrophion. The respiration, near the end, becomes slow and gasping—relieved by glucose.

Vomiting or lavage of the stomach always initiates recovery, and in 2 hours the blood NH_3 will be normal and in 6 hours the animal will have recovered.

If ammonium chloride be injected into the blood of normal dogs (0.35 gm. per kilo body weight) the blood ammonia will rise to 1.5 to 2 mgm. per 100 cc. associated with marked nervous symptoms. The ammonia gradually disappears from the blood and in 2 hours is all gone. The animal fully recovers in from 6 to 8 hours.

The symptoms in these two cases, while not identical, are similar, and the chemical findings in regard to NH_3 are identical. Taken together they strongly suggest that at least one of the causative factors in meat poisoning in Eck-fistula dogs is the absorption of ammonia from the stomach due to food decomposition. The cause of the inactivity of the stomach is unknown.

The excretion of chloride, urea, and water by the human kidneys. EDWARD F. ADOLPH.

Ambard has shown that there is a maximal or limiting concentration for urea and possibly also for chloride, in the urines of dogs and human subjects. He has found that these two concentrations represent approximately equivalent osmotic pressures.

The nature of the maximal concentration was studied upon a single human individual, over short periods of time. A dose of the substance whose excretory concentration was to be studied was ingested in the morning, and the urine was collected at approximately hourly intervals. The following determinations were made: volume, specific gravity, dry weight, chloride, urea, bicarbonate, and depression of the freezing point.

It was found that the greatest concentrations were obtained at the close of the salt diuresis, which usually lasted 7 or 8 hours. When either sodium chloride or urea was ingested alone, this substance constituted 50 to 80 per cent of the dissolved substances in the urine, and chloride and urea together constituted 80 to 90 per cent.

The maximal concentration for chloride and for urea is influenced by *a*, The length of time during which a large amount of the substances is excreted. The concentration increases 5 to 15 per cent. *b*, The amount of substance in the body at one time, and *c*, The amount of water available for carrying the substance through the kidneys. This change in urinary maximum is roughly proportional to the change in the osmotic pressure of the body fluids. Severe thirst increased the urinary maximum 10 to 20 per cent. There is considerable fluctuation in the maximal concentration; it increased 20 per cent in one year for the present subject. The chloride and urea concentrations, however, are always proportional.

Assuming that electrolytes are excreted ionized, it was found that the average maximal concentration for molecules plus ions of sodium chloride was 0.50 M. The concentration for urea was 0.65 M. The maximal concentrations for urea, chloride, and bicarbonate are osmotically equivalent. This may mean that the same mechanism is concerned in the excretion of all these substances.

Haldane found that bicarbonate is excreted in such a way as to form an additive maximum with chloride, and my experiments confirm this observation. Thus: Maximal concentration of chloride after chloride ingestion, 0.295 M, 0.297 M, 0.308 M. Maximal concentration of chloride plus bicarbonate after ingestion of both, 0.297 M, 0.307 M. The interference between chloride and urea is not so exact as that between chloride and bicarbonate. After ingesting large quantities of the former substances simultaneously, the following concentrations were found: 0.70 M, urea plus 0.20 M chloride, and a depression of the freezing point corresponding to 1.15 M. Any other substances excreted, no matter what their chemical character, served merely to depress the concentrations of these two constituents. There is a distinct limit to the total osmotic work which the kidneys can perform, and this is the true maximal concentration, rather than the concentration of any single constituent. The excretion of water is usually not influenced by the excretion of substances dissolved in it. But in concentrated urines the supply of water available for excretion is usually overdrawn.

The inhibition of erection by decerebration. E. G. MARTIN and M. L. TAINTER.

In dogs, anesthetized with ether or A. C. E. mixture, periphera stimulation of the intact N. erigens by means of shielded electrodes results typically in normal erection of the penis.

Transection of the brain stem at the level of the corpora quadrigemina abolishes the above reaction promptly and completely.

That the effect is not due to lowered blood pressure was proven by experiments in which arterial pressure was reduced by means of hemor-

rhage to the shock level without disturbing the onset of erection. The change in volume of the penis diminished as the blood pressure fell, but otherwise the reaction was typical. Intravenous injection of Ringer-Locke solution restored the full response.

Transection of the brain stem at any level involving the third ventricle is without effect on the onset of erection.

If both N. erigentes are cut or the lumbar cord is cut before transecting at the level of the corpora quadrigemina the onset of erection is not affected.

After the failure of erection has been brought about by decerebration repeated attempts to restore it by cutting the cord or the N. erigentes have failed except in a single experiment, in which section of the cord at the 2nd lumbar was followed by typical erection upon peripheral stimulation of the N. erigens.

That peripheral stimulation of the N. erigens was being accomplished in the experiments in which erection was not obtained was proven by the occurrence of marked unilateral contraction of the bladder at each stimulation.

The relative stimulating effect of light of different wave-length in an equal energy spectrum. HENRY LAURENS.

In investigating the effects of radiation the physical composition of the radiation should be taken into consideration, as well as the amount of response. Strict attention must be paid to the intensity and quality of the stimulus. Physiologists in general have disregarded these radiation factors.

In order to fulfil these conditions with a fair degree of accuracy, an apparatus was described in 1917 by the use of which the experimental conditions could readily be duplicated. This apparatus (spectrometer and radiometer) enabled one to obtain radiation of a specified small number of wave-lengths, the radiation being either of equal radiant power throughout the speetrum, or of known, variable energy content.

Using such bands of light the visibility of radiation has been determined by the method of flicker photometry. This is the relative sensibility of the eye to light (visible radiation) of different wave-lengths, and represents the ratio of the luminous intensity measured in light units to the intensity of the light measured in energy units. The curve is the luminosity curve of the human eye for an equal energy spectrum and is the curve which would be obtained by plotting the stimulating values of the spectrum of a source emitting equal amounts of energy at all wave lengths, if such an one existed. Visibility is determined by measuring the relative spectral energy necessary to produce a given luminous sensation throughout the visible spectrum. The reciprocal of this energy is then taken as proportional to the relative visibility.

Our visibility curve for high and low illumination is in essential agreement with that obtained by the numerous other investigators who have studied this function. It shows a maximum for high illumination at $555_{m}\ \mu$, for low illumination at $520_{m}\ \mu$, and slopes symmetrically to the

two ends of the spectrum. This determination of the visibility was primarily done as a check on the apparatus and our methods.

In addition to studying the relation between luminous sensation and radiant energy the pupillar response (direct reflex) to different wavelengths of equal energy has been studied in the alligator, pigeon, chicken and man. The record of the size of the pupil being made by photographing it (flash light).

The relation of the adrenals to fatigue. F. A. HARTMAN.

The denervated pupil (superior cervical ganglion removed) of a normal cat usually dilates until it becomes larger than the control, after working in a treadmill for a short time. This dilatation commonly increases as the work proceeds. After removal of both adrenals this "fatigue" dilatation is abolished. Even after cutting the splanchnic nerve to a single remaining adrenal, the "fatigue" dilatation is greatly reduced or abolished. Such dilatation, therefore seems to be due to activity of the adrenals, an increase in the output of epinephrin.

Cats, possessing but a single adrenal and that denervated are not able to work so hard or to travel so far as normal animals, moreover such work is accompanied by little or no dilatation of the denervated pupil. Some of these animals are seized by convulsions if they work hard. After ample time has elapsed for regeneration of the nerve fibers to the adrenal the same animal shows greater dilatation of the pupil, can travel farther and convulsions either entirely disappear or are brought on with difficulty.

Fatigue convulsions occasionally occur in normal cats but they are rare and are never preceded by a good dilatation of the denervated pupil. The same animal will work faster and go farther when there is a good dilatation of the denervated pupil.

Our study of the denervated pupil reaction in normal cats and those possessing but a single adrenal and that denervated indicates that epinephrin increases the ability to work and to withstand fatigue.

Calorigenic effect of adrenalin in dogs. WALTER M. BOOTHBY and IRENE SANDIFORD.

It has been shown that the administration of 0.5 mg. adrenalin when injected subcutaneously in man causes an increase in the rate of heat production; the height of this reaction is about 10 minutes after the injection and lasts about 2 hours. It is impossible to calculate the exact rate of absorption of the adrenalin into the blood stream but probably it never exceeds rate of 0.5 mgm. in 5 minutes which in a 65 kilo individual is at the rate of 0.0016 mgm. per kgm. per minute. Stewart and Rogoff have shown that under normal experimental conditions from 0.0003 to 0.001 mgm. per kilo per minute of adrenalin are normally liberated. Cannon and Rapport have shown that by reflex stimulation in cats as much as 0.0032 to 0.0037 mgm. per kilo per minute can be secreted at least for short intervals. Stewart and Rogoff in a discussion of our paper last year maintained that we were using doses that

could not be even approximated in life in spite of the calculation given above.

To investigate this matter further we have carried out with Dr. Mann's help a study of the effect of adrenalin in dogs under urethane anaesthesia by injecting the drug both subcutaneously and intravenously and we have found that a barely noticeable calorigenic reaction is produced by the subcutaneous injection of 0.2 cc. = 0.016 cc. per kilogram and with the maximum effect occurring between 5–10 minutes afterwards the rate of absorption per minute was probably not far from 0.0016 to 0.0032 mgm. per kilo per minute.

By intravenous injection of 0.5 mgm. in 49.5 cc. of salt solution over a period of 10 minutes which is at the rate of 0.0045 mgm. per kilo per minute we have produced a very definite calorigenic effect of between 10 and 40 per cent as shown on the curves. The reaction was carefully controlled both by trained observers and by kymographic records and we feel that the increased heat production was not caused by movements but was due to the specific calorigenic power of adrenalin. No effect was produced in one experiment by the intravenous administration of a very small dose amounting to 0.0008 mgm. per kilo per minute. The comparison of our doses with those obtained under normal conditions by Stewart and Rogoff and that obtained under adrenal stimulation by Cannon leads us to conclude that probably the adrenal glands can spontaneously discharge sufficient adrenalin to materially affect the rate of heat production. In consequence the mechanism by which adrenalin produces its various physiologic effects may be a direct chemical stimulation of cellular activity; different cells and organs may have a different concentration threshold of activation and thus explain the reversed effects produced by different doses. Our data support Cannon's broad hypothesis of the "emergency action" of the adrenal glands.

The gasometer method of indirect calorimetry for dogs. WALTER M. BOOTHBY and F. C. MANN.

Indirect calorimetry has become widely used in the clinic, many questions that can only be solved by animal experimentation have arisen as the result of our more extended knowledge of the basal metabolic rate and the respiratory quotients and the variations in health and disease. In the next few years many physiological laboratories will be undertaking problems of this nature and therefore the question of the choice of method for animal work is important.

As a result of the experience obtained in human work we were able to adapt readily the gasometer method to dogs. A delicately balanced gasometer of a volume of 125 liters is used and arranged with inspiratory and expiratory valves of the flutter type enclosed in glass. The dog should be made comfortable on a flat table covered with a mattress and the animal kept warm with blankets if necessary. The dog's neck is shaved and a few turns are made around it with a 5 inch rubber dental dam, taking care to have it smooth and snug and yet not causing

undue pressure; a copper mask with inlet and outlet tubes at the dista end is slipped over the dog's head and held securely in place by four tapes tied to a tape encircling the dog's neck outside the rubber dam; after these have been fastened a few more circular turns of the rubber dam are made over the tapes and down on the copper mask; with a little practice the connection can be made absolutely air-tight without any discomfort to the dog. Graphic records of any movements are recorded by pneumographs over the chest (upper extremities) and abdomen (lower extremities), and simultaneous notes of the dog's condition, pulse and respiration should be made by the observer. The original readings of the gasometer should be made in duplicate from two pointers and checked by the assistant; the time must be taken by a stop watch and the readings checked by the assistant and the correctness of the minute hand reading controlled by an independent record of the exact time of the beginning and ending of the experimental period by an ordinary watch. The expired air must be analyzed in duplicate on a Haldane gas analysis apparatus which has been carefully calibrated; the accuracy of the Haldanes and of the analyst must be carefully controlled by repeated outdoor air analyses, records of which should be kept. The calculations must be checked.

In our opinion the gasometer method has a more general application and is technically easier than the closed circuit box method and is particularly adapted to a series of experimental periods of short duration both for the determination of variations of metabolic rate and the respiratory quotients.

Effect of cocaine on the growth of lupinus alba: A contribution to comparative pharmacology of animal and plant tissues. D. I. MACHT and MARGEURITE LIVINGSTON.

The effects of cocaine and its decomposition products were studied on the growth of seedlings of the plant *lupinus alba*. The seeds were soaked in water and allowed to sprout in a suitable medium, following which the length of the straight roots grown by this plant was measured and the effects of cocaine and other chemicals on the growth of the roots were investigated. The plants were placed in solutions of nutrient salts (Shive solution) and the various drugs were added to such solutions. Controls were made on seedlings suspended in Shive solution diluted one-half with distilled water. The effects of cocaine and its decomposition products on the growth of lupinus are very different from the effect of the same substances on animal tissues. Whereas cocaine is very toxic for animal tissues such as smooth and skeletal muscle, nerves, etc., it required strong solutions of this alkaloid, namely 2 per cent of cocaine hydrochloride, to inhibit the growth of the seedlings. Ecgonin hydrochloride inhibited growth in concentrations of 0.0055 per cent, while benzoyl ecgonin was much less toxic requiring $2\frac{1}{2}$ per cent concentration to affect the growth. Methyl alcohol was found to be very little toxic to the roots of the lupinus, requiring 4.8 per cent to produce an inhibition of growth. The most

toxic decomposition product of cocaine was found to be sodium benzoate, a compound which is practically non-toxic for animal tissues. Sodium benzoate was found to be deleterious to the lupinus root in concentrations of 0.007 per cent; while the ester methyl benzoate produced inhibition in concentrations of 0.014 per cent. Various simple mixtures of ecgonin, sodium benzoate, methyl alcohol, benzoyl ecgonin, etc., were also studied.

The production of carbon dioxide by the smooth muscle of sea-anemones. G. H. PARKER.

The common sea-anemone Metridium will contract its muscles to about one-tenth their full length and remain thus contracted for a day or more. The amount of carbon dioxide produced by a contracted sea-anemone is about the same as that produced by it when it is relaxed. Nor does this amount change perceptibly during the process of relaxation, but during the operation of contraction the output of carbon dioxide is increased by a fourth or more. Thus a special consumption of energy takes place only during the act of contraction. The contracted condition is maintained without any unusual metabolic change and may depend upon some form of "catch" mechanism in the muscle as is supposed to be true of the catch muscle in the Pecten.

The rôle of the sodium ions in the contraction of the isolated duodenal segment of the albino rat by sodium carbonate. F. S. HAMMETT and J. E. NOWREY, JR.

The contraction of the isolated duodenal segment of the albino rat which follows the addition of tenth molecular sodium carbonate solution to the oxygenated Tyrode's solution in which the segment is suspended is due neither to the increase in the sodium ions nor to the increase in the sodium calcium ratio. This conclusion is drawn from the fact that the addition to the solution in which the segment is suspended of an equal amount of sodium in the form of sodium chloride, such as produces a contraction when added in the form of sodium carbonate, fails to produce a contraction. While it is true that the increase in the sodium ions may participate in the effect by increasing the permeability of the tissue for the agent initiating the reaction, this increase in permeability can not be considered as the primary cause of the contraction.

The rôle of the change in hydrogen-ion concentration in the motor activities of the small intestine. FREDERICK S. HAMMETT.

When that amount of sodium hydroxide is added to Tyrode's solution in which there is suspended an isolated intestinal segment from the adult, male, unexcited albino rat, which produces the same hydrogen-ion concentration as develops on the addition to the same amount of Tyrode's of 0.2 cc. of tenth molecular sodium carbonate solution, the segment responds by a shortening approximately equal in degree to that obtained with the carbonate application. It is therefore con-

cluded that the stimulus to this contraction is not due to the increase in carbonate ions but is due to the increase in the hydrogen-ion concentration. On the basis of these and previous observations there is developed the theory that in the normal undisturbed organism during digestion the changes in the hydrogen-ion concentration of the intestinal contents determines the intestinal motor activities which are superintended and directed by the intestinal neural mechanisms.

The heart of hibernating animals. JOHN TAIT.

Whereas the excised perfused heart of an ordinary mammal ceases to beat at a temperature of about 17°C., that of hibernating animals (hedgehog, woodchuck) will beat at very low temperatures. The woodchuck heart ceases to beat only when it freezes. At very low temperatures its action is fully coördinated, the extent of its contraction is as great as before, but its rate is exceedingly slow, 2 per minute. Cooled through a range of 40° its rate is slowed to one sixtieth or one seventieth. A phrenic-diaphragm nerve-muscle preparation from hibernating animals continues in activity at relatively low temperatures. Apparently certain tissues of such animals are adjusted to function at a different temperature level from those of other animals. It is suggested that the difference may be connected with a difference in the solidification point of the contained lipoids.

Physical fatigue and susceptibility. ELLA HUTZLER OPPENHEIMER and REYNOLD A. SPAETH.

A fatigued individual is generally believed to be more susceptible to disease than a non-fatigued individual. We have carried out a series of experiments to determine whether a state of fatigue will produce in experimental animals a greater susceptibility to toxins and infections. By "fatigue" we mean a condition of temporary exhaustion produced by excessive muscular exertion.

Our experiments were on a carefully selected cross-bred stock of hooded and white rats. These animals were kept at a uniform temperature on a constant McCollum diet. Throughout the period of experimentation no animal was lost from extraneous infection. So far as possible brothers and sisters or cousins were used as controls for the fatigued animals. The following results were obtained:

1. Fatigue, artificially induced in white and hooded rats by forcing them to run in motor driven drums, apparently tends slightly to increase their resistance to subcutaneous injections of tetanus toxin. This occurs whether fatigue precedes or follows the injection of the toxin.

2. White and hooded rats, up to 185 grams in weight, are not susceptible to diphtheria toxin in doses as large as 2 cc.

3. Fatigue, both preceding and following intraperitoneal injections of type I pneumococcus, definitely increases the resistance of the white and hooded rats to that infection.

4. Our results do not confirm the generally accepted conception of

the relation between fatigue and "natural susceptibility," in contrast to the observations of Charrin and Roger (1890) on white rats injected with anthrax, and Abbot and Gildersleeve (1910) on rabbits injected with streptococcus pyogenes.

The distribution of the vagus nerves to the sino-auricular junction of the mammalian heart. GEORGE BACHMANN.

The sino-auricular junction is represented in the adult mammalian heart by the line of attachment of the right venous valve. This line is along the crista terminalis and the base of the Eustachian valve. In animals in which this structure is absent, the tissue above the mouth of the coronary sinus as far as the limbus of the fossa ovalis represents the line of attachment of the Eustachian valve.

It has been known since the discovery of the S-A node by Keith and Flack that numerous nerve cells and fibers related to the vago-sympathetic are found near the node. A microscopic examination of two interauricular septa of the dog's heart, revealed the presence of several ganglia in the tissue between the mouth of the coronary sinus and the limbus of the fossa ovalis. Many ganglia are found on the mesial (left) side of the superior vena cava. Ganglia are found also in the mass of fat situated between the two venae cavae (intercaval angle). Ganglia pass for a short distance from this angle into the connective tissue of the tubercle of Lower.

An attempt was made in fifteen dogs to destroy the nerve cells and fibers in the various areas mentioned. The destruction was accomplished by injecting these areas with a solution of potassium dichromate, acetic acid and formalin. The right and left vagi were stimulated with a definite strength of current previous to the injection, and similarly after the injection of each of the areas experimented upon. The changes in the chronotropic effect of vagus stimulation were estimated in percentages and from these the percentile loss of chronotropic influence computed. Changes in dromotropic effects between auricles and ventricles on stimulation of the vagi were also noted.

The S-A node is supplied by both nerves; the head of the node predominantly by the left nerve, and the tail of the node predominantly by the right nerve. The right vagus predominance on the tail is, however, greater than the left vagus predominance on the head of the S-A node. Hence it is that a strength of stimulus that is sufficient to lead to a standstill of the auricles when applied to the right nerve, is often inadequate for this purpose when applied to the left nerve.

The coronary sinus area was supplied by the left vagus in nearly every instance in which successful results were obtained. The median (left) side of the superior vena cava is supplied nearly to the same extent by both nerves. The intercaval angle is supplied by the right vagus. The changes in the dromotropic effects of vagus stimulation will be described later.

On the increased weight of spermatozoa in egg-secretion. OTTO GLASER.

The spermatozoa of Arbacia settle more rapidly in egg-secretion than in sea-water. This is not attributable to differences in the salt content of secretion and sea-water, nor to dehydration of the sperm through abstraction of water by the secretion. The phenomenon is explainable as the result of an increase in weight. This increase, apparently, is not physical, but rather the outcome of a chemical combination between a sperm-borne valence of some kind and some constituent of the egg-secretion. The basis for this belief is the fact that an acceleration of the settling-rate occurs only when the egg-secretion is sufficiently concentrated; and, that acceleration in properly concentrated secretion does not occur if the suspected chemical union is prevented. In this connection, the agglutinin is considered the important constituent of the secretion since the settling-rate is not accelerated in artificially prepared agglutinin-free egg-secretion.

Studies on the effects of x-rays upon glandular activity: I. The submaxillary gland. A. C. IVY, B. H. ORNDOFF and A. JACOBY.

The acute and chronic effects of x-rays upon the submaxillary gland have been studied in dogs. The chronic effects were studied on dogs with a Wharton's duct fistula. The acute effects were followed under light ether anesthesia by cannulating the ducts on both sides and exposing the chorda-lingual nerves for stimulation with currents of equal strength, and subjecting the gland on one side to x-rays of varying intensities, using the other gland for a control. The effect of x-rays on the volume flow of blood was also observed in some animals. The chemistry of the secretion was studied before and after exposure of the gland to x-rays.

In animals with a salivary fistula observations were made *a*, on the normal variations in the continuous secretion; *b*, the response to acid (0.5 per cent HCl) applied to the oral mucosa; *c*, the response to chewing meat for 2 minutes; and *d*, the response to hypodermic injection of 5 mgm. of pilocarpine hydrochloride.

It was found in confirmation of Pavlov and his co-workers, that an x-ray dosage of 50 K. V. Peak, 8 ma. current, no filter, 30 cm. focal distance, 6 cm. portal of entry, applied for ten minutes had no effect upon the gland, neither stimulating, sensitizing nor depressing. A dosage of 100 K. V. Peak, 5 ma. current and the above mentioned "set-up" applied for ten minutes had a differential depressive effect after four exposures. A dosage of 147 K. V. Peak, 5 ma. current and the above "set-up" applied for ten minutes had a more marked effect, but still differential, than dosage of 100 K. V. Peak. The differential effect referred to is a reduction of forty per cent in the amount of secretion excited by chewing meat and by pilocarpine injection, the response to excitation by oral application of acid not being altered in amount.

The chemical analyses show a marked reduction in the total solids of the secretion associated with a marked reduction in the viscosity, the inorganic solids being reduced more than the organic solids.

In some of the acute experiments the weaker dosage caused a depression in the salivary response excited by chorda lingual stimulation, while in others the second dosage was required to produce depression. Stimulation, or even sensitization, has not been observed by us.

Variations in muscular efficiency in women. LILLIAN M. MOORE and J. LUCILE BARKER.

The purpose of this investigation is to determine the daily efficiency of women over a period of several months, in order to show whether the efficiency remains fairly uniform or varies periodically, according to the fundamental rhythm responsible for the monthly cycle.

Tests of muscular efficiency were made as an index to general efficiency. The Martin spring balance dynamometer, which measures the breaking and not the pulling strength of the muscles, was used. Eight muscle groups, arm flexors, thigh abductors and adductors and pectorals were tested. The total muscular strength of the body was calculated from these data. Eighteen subjects were used over periods varying from 2 to 14 months. The tests were given daily at the same hour for each individual. Sundays and occasional other days were omitted. The subjects were college students and young instructors. In addition to the strength records data were kept of the number of hours sleep, general physical and mental condition, and routine of the previous day.

Results were plotted with time in days as abscissae, and total muscular strength in kilograms as ordinates. Only a limited number of the resulting graphs show an unmistakable rhythm, periodicity if present being masked in many by the unavoidable daily fluctuations often of considerable magnitude. Composite curves were made for each individual by superimposing the data for each monthly cycle and averaging the identical phases of all the cycles bring to light periodically recurring variations. These are constant for all the cycles, while the daily variations are scattered indiscriminately throughout the different cycles. The curves were smoothed in the usual way, by averaging each phase value with the two adjacent phase values.

The smoothed composite curves, with two exceptions, display the following characteristics; two periods of high and two of low efficiency varying in extent and duration. In eleven the point of highest efficiency appears in the premenstrual, and a second of less magnitude in the post menstrual period with two of lowered efficiency between these. In five the post menstrual rise is greater. The two points of depression are nearly equal. With one exception, the strength is below average at least one day during the menstrual period, but the lowest point is apt to occur in the second depression.

Composite curves were made for each half of the individuals, after changing the strength data to per cents so that each will contribute equally. These two composite curves of individuals chosen at random are practically identical. The probable error of the curves of 0.00917, means a chance of 1 in 1000 that the variations are accidental and

not periodic. A composite curve from the 18 individuals was made by averaging these two. This curve includes data from 1517 tests taken throughout 100 monthly cycles. It shows the typical rhythmic variations in muscular efficiency, that is, a gradual rise from the beginning of the intermenstrual to a maximum just prior to the menstrual period as the significant variation, a depression during menstruation as a secondary phase of less magnitude, and a short period of increased efficiency in the post menstrual period.

Relation of number of hours sleep to muscular efficiency. LILLIAN M. MOORE, LULA MARIE JENKINS and LUCILE BARKER.

In the experiments demonstrating rhythmic variations in muscular efficiency in women, it was noted that many of the chance variations superimposed upon the rhythm were caused by alterations in the number of hours sleep during the previous night. Occasionally the effect was apparent on the second day instead of the first.

Analysis of the data which included about 1500 tests on 26 individuals revealed a rather uniform correlation in most cases. However, since the variations in the number of hours of sleep were entirely fortuitous, and the activities of the subjects were not controlled, the sleep effect in many cases was masked by the influence of various mental and physical disturbances, such as examinations, social activities, etc. Whenever the number hours sleep was the only variable, the results were similar, that is, muscular efficiency in kilograms, as measured by the Martin spring balance dynamometer, increased or decreased as the number of hours sleep increased or decreased within certain limits. The usual range of hours was 6 to 9 inclusive.

These results, while not conclusive, were sufficiently suggestive to lead to a series of controlled tests on 5 subjects, tests which are still in progress. In these the conditions and activities of the subjects are kept as constant as possible without disturbance of the necessary normal routine, the only noteworthy variable being an increase or decrease in the number of hours of sleep. In the results thus far obtained a definite correlation is shown in every subject. The correlation table of one subject shows a gradual increase in strength, corresponding to the increase in hours sleep. A graph with strength in kilograms as abscissae, and hours sleep, 6–9 hours as ordinate, shows the same thing. Data for this subject give a calculated correlation of 0.5.

The regulation of respiration. F. H. SCOTT, C. C. GAULT and R. KENNEDY.

That the expansion of the thorax in inspiration stimulates the sensory nerves of the thoracic cage can readily be shown by laying the peripheral end of a cut intercostal nerve over electrodes and connecting to a string galvanometer. As the thorax expands electrical variation may be seen in the nerve. In a previous communication the effect of section of the cord and section of the thoracic posterior roots was studied in cats. We concluded the loss of the sensory impulses from the thoracic

cage affected the centre in an antagonistic manner to the sensory impulses of the vagus. While this is the usual result in a number of cases we have done this operation without any alteration in respiration.

Since section of the cord produces a great lowering of blood pressure the effect of altering blood pressure on respiration was investigated. Lowering of the blood pressure by stimulation of the peripheral vagus or by gravity caused a slowing of respiration while lowering of the blood pressure from splanchnic section caused an increase in respiratory rate.

Some relations of the vagus and the spinal afferent nerves in respiratory control. F. H. PIKE and HELEN C. COOMBS.

Scott's statement (Journal of Physiology, 1908, xxxvii, p. 301) that the afferent nerves from the muscles and other parts of the respiratory mechanism have much the same rôle in the control of respiratory movements that other somatic sensory nerves have in the control of movements of skeletal muscles generally directs attention to the detailed study of the afferent tracts involved. Mathison (1912) showed that afferent fibers in the phrenic nerve were to be included in this list. The rôle of the afferent fibers from the thoracic wall was still unexplained. Stewart (1907) in dogs and Schäfer (1919) with cats found that the respiratory rate returned to normal in certain instances after bilateral vagotomy.

We have divided both vagi simultaneously in cats, without cauterization. Some of them recovered, but there was little or no recovery of the normal respiratory rate. When the animals were again anesthetized after an interval varying from six to eighteen days, and the dorsal roots of the thoracic nerves divided, the respiratory movements were disturbed just as seriously as when the whole procedure was carried out at the same time. This holds also for transection of the brain stem just below the corpora quadrigemina, without lesions of the dorsal roots. The dorsal roots of the thoracic and cervical nerves appear to be the afferent pathways for the regulation of respiratory movements after section of both vagi.

From our experiments, we believe that at least four factors are concerned in the control of respiratory movements: *a*, The concentration of carbon dioxide or other substances in the blood; *b*, the temperature of the blood; *c*, the volume of blood flow through the medulla oblongata in unit time; and *d*, afferent nerve impulses over the vagi, the phrenics and the dorsal roots of the thoracic nerves. Afferent impulses from other regions may be involved under certain conditions. Summation of the action of these various factors seems to occur in the respiratory cells of the bulb. We consider the action of the first and fourth factors particularly to be a case of summation of stimuli.

Voluntary stimulation of sympathetic nerves. N. B. TAYLOR.

The subject of this investigation is a student of medicine, aged 26, of robust physique and of a somewhat phlegmatic temperament. He

is capable at a given sign to increase immediately the rate of the heart from 70 to 125, an increase of 55 beats per minute. The acceleration can be maintained for several minutes and can be brought to an immediate termination at will. The acceleration is not preceded or accompanied by a self-induced emotional state of any sort but is produced through a certain exercise of the will, the precise nature of which he is unable to describe.

The administration of atropine $\frac{1}{30}$ of a grain hypodermically did not abolish the voluntary control over the heart though it did disturb it quite definitely. The rate was increased to 90 beats per minute by atropine. The greatest acceleration attained by voluntary effort while under the influence of the drug was 116, this being an increase of only 26 beats per minute over the existing rate. The blood pressure was elevated, the systolic pressure rose from 118 before to 150 mm. Hg. during the paroxysm. The diastolic pressure did not rise to the same relative degree as the systolic, so that the pulse pressure was increased considerably during acceleration. Peripheral vaso-constriction was associated with the change in heart rate. The blood-flow through the hands, as estimated by Stewart's method was markedly curtailed, falling in one instance from 7.8 grams before to 2.9 grams per 100 cc. of tissue per minute during the acceleration. Accompanying the cardiac and vascular changes were dilatation of the pupil, muscular twitchings and a slight increase in the respiratory rate. It was shown however that neither of the two last mentioned phenomena was a factor in the production of the acceleration. Glycosuria invariably occurred after a series of paroxysms. A series of tests was carried out in which the subject's urine was examined upon three successive days during which no acceleration was undertaken and on a day upon which the heart was repeatedly accelerated. The diet during the entire period of 4 days was kept constant in regard to its content of carbohydrate. Though no sugar could be detected in the urine by qualitative estimations (Benedict's) on the three normal days, pronounced glycosuria (0.5 per cent–0.6 per cent) occurred upon the day during which the heart was accelerated, the sugar making its appearance 2 to 3 hours after the last paroxysm and persisting for 2 hours. Estimations of blood sugar were made throughout the series but in no case was any definite alteration in the sugar level detected.

The cathode ray oscillograph as a means of recording nerve action currents and induction shocks. H. S. GASSER and J. ERLANGER.

The cathode ray oscillograph or Braun tube is a device in which a pencil of cathode rays is made to impinge upon a fluorescent screen. This stream may be deflected instantaneously by an electrostatic or electromagnetic change in the field through which it passes. The form of the tube used is one, devised by J. B. Johnson,[1] which has the particular advantage that the rays are slow and easily deflectable. The electrons, formed at a hot cathode A, are pulled toward the anode,

[1] Am. Physical Soc. Proc., Phys. Rev., 1921, xvii, 190.

B, by means of a 300 volt potential supplied by small dry cells. The electrons shoot through the anode which is tubular and past the deflecting plates, *C*, which are in pairs and at right angles to each other.

One of the pairs of plates is connected to a potentiometer. This consists of a circular resistance, *D*, against which a brush revolves at 30 revolutions per second and across which there is a 150 volt potential drop. As the angular velocity is constant the spot is moved along the abscissa at a constant velocity of about 2 meters per second. On the shaft which carries the brush is a rotating interrupter inserted in the primary of an induction coil. To record the form of the induction shock, the secondary of the induction coil is connected to the second pair of plates. The make and the break shock therefore move the spot along the ordinate. The result is that the shock is plotted as a standing wave in rectangular coördinates of which the abscissa is the time and the ordinate is the potential of the shock.

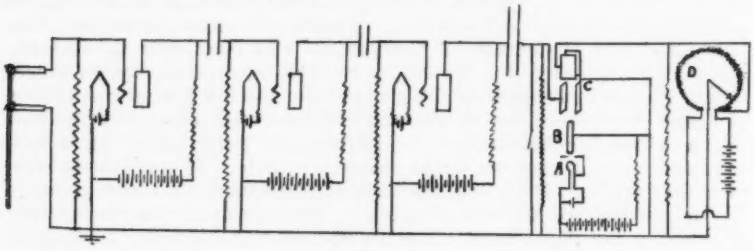

Fig. 1

To record the action current of nerve, frog sciatic was used. Leads were made so that the action current would be monophasic. As 22.5 volts are necessary to produce 1 cm. of deflection of the spot, the electrodes were connected to a three stage amplifier giving 3000 fold amplification and the output of the latter was connected through a 20 m.f. condenser to the deflecting plates of the tube. The result was the plotting of an action current of striking clearness completed as to its quick part in less than 20σ.

The green spot quite brilliant to the dark adapted eye is not very active on the photographic plate. Therefore, the most satisfactory method of recording has been to trace the pattern on the tube itself. By holding a film against the tube a contact print can be made. Lantern slides were presented of such prints and of action current and induction shock tracings.

The electrical resistance and reactance of suspended unicellular organisms. S. C. Brooks.

Unicellular organisms (*Bacillus spp.*, *Chlorella*, *Saccharomyces*) were suspended in balanced salt solutions, and centrifugalized at intervals. The difference between the resistance of the supernatant liquid and that of the suspension resulting when the cells were stirred up was

expressed in per cent of the former, and called the net resistance in per cent. Net capacitance in per cent was derived similarly, and was consistently negative in sign. This is probably due to the lower mean dielectric constant of the organism. For normal cells net resistance % 1 net capacitance % = const. = 1 within the limits of error of the method. The same ratio obtains during a considerable part of the process of death, as in the presence of acid. There is evidently an intimate relation between the two properties.

The net resistance of dead cells is relatively much greater than in such multicellular forms as *Laminaria*.

Survival curves show that under certain conditions the net resistance is a function of the proportion of viable cells present.

Pseudo-paradoxical pupil-dilatation following afferent path lesions. JOSEPH BYRNE.

After afferent nerve injuries, mechanical or toxic-infective, one pupil becomes larger than its fellow. The phenomenon resembles true paradoxical dilatation but has a different mechanism. Hence the name. After unilateral section or crushing of the middle division of the fifth cranial nerve, or of the brachial plexus, or of one or more cervical nerves the phenomenon appears in the homo-lateral pupil. After sciatic nerve lesions it appears in the contra-lateral pupil. It occurs preeminently in the period preceding, and subsequent to, the true paradoxical period. In the latter it is usually, though not necessarily, absent. The mechanism consists of the hyperfunctioning of slightly injured neurones in the preparadoxical period (over-stimulation) and of more seriously injured neurones in the post-paradoxical period (repair). The associated hyperalgesia indicates the prominent rôle of the nociceptive neurones.

The catalase content of normal and atrophied muscles. A. E. GUENTHER and S. MORGULIS.

The catalase content was determined by the method described earlier (Morgulis, J. Biol. Chem., xlvii, 341; This Journal, lvii, 125, 1921). The experiments were made on rabbits, the muscles of one limb undergoing atrophy owing to section of the sciatic nerve. The animals were killed by decapitation. The muscles (extensor longus digitorum) from both the normal and atrophied limb were taken intact, quickly weighed and ground with a small amount of pure sand. The ground material was then extracted with chloroform water, the catalase determinations being made on measured portions of the filtered extract.

All determinations were carried out under uniform conditions so far as volume of the mixture, temperature, reaction (pH = 7.0) and rate of shaking are concerned. Using an amount of hydrogen peroxide sufficient to yield 50 cc. of oxygen gas, the quantity of extract was found which was necessary to bring about a 70 per cent decomposition.

Since all the extracts were made up to 100 cc., it was a simple matter to calculate the catalase capacity of the total extract and therefore of the entire muscle.

Data recorded in the table show that the atrophy of the muscle was far advanced, the denervated muscle having lost about fifty per cent of its mass. However, while the muscle substance was progressively diminishing in weight though retaining its physiological efficiency (Peters and Guenther, Journ. Biol. Chem., xlvi, 1, 1920) the total catalase capacity evidently remained unaffected by the processes of atrophy. An examination of the figures in the third column reveals that in rabbit I the total catalase capacity of the normal muscle is somewhat higher than that of the atrophied but that the reverse is the case in rabbit III, while in rabbit II the catalase content of both normal and atrophied muscle is practically the same. This leads to the conclusion that the catalase in the muscle is confined in such a way that it does not autolyze and diffuse into the blood stream as has been shown to be the case with other components of the protoplasm. The catalase evidently belongs to the more stable constituents of cell substance.

ANIMAL		WEIGHT OF MUSCLE	CATALASE EXPRESSED IN CUBIC CENTIMETERS OF OXYGEN LIBERATED	
			Entire muscle	One gram of muscle
I	Normal	1.88	165	88
	Atrophied	0.96	152	161
II	Normal	2.27	106	43
	Atrophied	1.12	103	92
III	Normal	1.43	109	76
	Atrophied	0.73	133	182

The fact merits further consideration that the catalase capacity per gram of muscle substance varies inversely as the weight of the muscle. Thus, in rabbit I, II, and III with a relation of the atrophied muscle to the normal of 0.5, the ratios of the catalase content per gram of substance are 1.83, 2.14 and 2.40 respectively. This shows the fallacy of attempting to express the catalase capacity or activity per unit of weight of an organ irrespective of the weight of the entire organ. As the catalase is one of the parts of the muscular substance which does not diminish in atrophy, its relative amount will increase in the same proportion as the total mass of the muscle is reduced by the atrophic changes. The muscle grows lighter owing to loss of various substances but as the catalase is not affected by this process the catalase activity appears to be greater.

The mode of action of physical work, cold weather, and cold baths in increasing the oxidative processes. W. E. BURGE.

Students, rabbits and dogs were used in the experiments.

The physical work consisted of gymnasium exercises for the students and running in a revolving wheel for the rabbits. For the cold baths

water at 5°C. was used and the students as well as the dogs remained in it for 5 minutes.

Catalase determinations were made of the blood immediately before and after the gymnasium exercises and the cold baths. A number of rabbits were kept under observation during the summer, fall and winter months and catalase determinations made of the blood during these seasons.

The catalase determinations were made by adding a known quantity of blood to neutral hydrogen peroxide in a bottle, and the amount of oxygen liberated in ten minutes was taken as a measure of the catalase content of the blood.

It was found that physical work as well as cold baths greatly increased the blood catalase. It was also found that the blood catalase was lowest in the hot summer months, moderately increased during the fall months and greatly increased during the winter months.

The increase in oxidation brought about by physical work, cold baths, and cold weather is attributed to the increase in catalase.

The relative alcohol content of blood and urine. W. R. Miles.

Ethyl alcohol introduced into the stomach begins quickly to be absorbed, to appear in the blood and to be utilized in the metabolism. The content in the blood gradually rises to a maximum and then more slowly decreases, reaching the base line some hours after ingestion. Widmark and Ambard claim that the alcohol content of the urine is identical with that of simultaneous blood samples and that the kidney does not concentrate it.

Experiments are described in which men, abstinent, moderate and habitual drinkers, without food, took 27.5 grams of absolute alcohol, in one instance diluted to 1000 cc. and on another occasion diluted to 100 cc. Urine samples were passed every 15 or 20 minutes and with certain of these, blood samples from an arm vein were taken 20, 40, 70 and 120 minutes following ingestion. The results with different subjects are very concordant. The alcohol concentration in the blood is at first above that of the urine and characteristically crosses the urine curve 25 to 30 minutes after ingestion. With the dilute alcohol (1000 cc. of 2.75 per cent by weight) the blood 60 to 120 minutes after drinking shows about 0.30 mgm. per cc. while the urine gives 0.45 mgm. per cc. and the plasma gives 0.36 mgm. per cc. The urine is therefore 50 per cent higher in alcohol content than the whole blood and 25 per cent higher than the plasma. The concentrated potion (100 cc. of 2.5 per cent by weight) gave averages 60 to 120 minutes after ingestion: blood 0.40, plasma 0.47 and urine 0.57 mgm. per cc. The plasma is 20 to 25 per cent higher than the whole blood. The concentrated alcohol is absorbed decidedly more rapidly than the dilute and in "habituals" much more rapidly than with moderate drinkers.

The determinations in duplicate were made by Widmark's modification of the Nicloux method. The procedure was identical for blood, plasma and urine, and "knowns" made with water, urine and blood

gave an average error of ± 3.6 per cent. No subject was found who demonstrated elimination by diffusion. The alcohol content of the urine only approximately indicates the form of the alcohol curve for the blood, and the kidney, also with this substance, follows its usual action of concentration.

What are viscera? C. JUDSON HERRICK.

The existing confusion in usage regarding the visceral neuromotor apparatus can best be ameliorated by adopting a frankly physiological definition of viscera and applying it uniformly in both physiological and anatomical description. Viscera are concerned with the nutrition, internal regulation and reproduction of the body, in distinction from the somatic organs whose reactions are externally excited and involve a change in the relations of the body or its members to environmental conditions. Visceral reactions are interoceptive; somatic reactions are exteroceptive; proprioceptive neuromotor mechanisms are subsidiary to the other two. Some organs perform both kinds of function and are of mixed character both physiologically and structurally. Organs which were primitively visceral but have secondarily acquired somatic or mixed functions can best be classed in accordance with their genetic relationships. The visceral nervous system comprises all neurons primarily concerned in visceral reactions, whether their cell bodies lie peripherally or centrally. The ancient definition of the sympathetic nervous system should be retained, viz., a dissecting room term for those visceral ganglionic plexuses which can be separated in the gross from the cerebro-spinal system. The term autonomic nervous system should be dropped. The visceral neuromotor organs are of two sharply distinct kinds: *a*, general visceral, whose efferent path is broken by synapse into preganglionic and postganglionic neurons; and *b*, special visceral, those visceral efferent paths composed of a single neuron and those afferent paths whose receptors are organs of special sense (taste buds and olfactory organ, in part).

Observations on cerebellar stimulations. F. R. MILLER and F. G. BANTING.

Whether the cerebellar cortex is excitable is disputed. Horsley and Clarke assert that it is inexcitable and that the effects yielded by stimulation are due to spread of current to the intracerebellar nuclei. Beck and Bikeles and others also consider it inexcitable. The belief that the cortex is excitable is, however, held by Rothmann and Shimazono. The results of stimulation recorded by different observers are very discordant probably due, in part, to the vitiation of results by escape of current to the accessorius and trigeminus nerves.

We stimulated the cerebellar cortex in the decerebrate cat and used the inhibition of decerebrate rigidity as a criterion of excitability of the cortex. The evidence which we believe establishes the excitability of the cortex is as follows:

a. The currents which evoke the inhibition are weak and the electrodes may be placed in the midline of the vermis, hence possible influence of

spread of current to the trigeminus or cut dural margin may be excluded. Furthermore, cocaine applied to the edge of the dura does not modify the inhibitory response.

b. The inhibition can only be evoked when the cortex is normal and "healthy looking," anaemia prevents it. Hence, it is not due to current spread to the intracerebellar nuclei.

c. The threshold for the inhibition is lower than that for the motor cerebral cortex.

d. Cocaine, which acts superficially, abolishes the inhibition.

The inhibition is elicitable from the greater part of the vermis anteroposteriorly. From the anterior surface of the lateral hemisphere the inhibition affects all the limbs but most intensely the ipsolateral hind leg. Unipolar faradisation of the superior peduncle (brachium conjunctivum) causes claw movements in the ipsolateral foreleg; these movements resemble those seen in a stroked cat.

Walking movements in the forelegs and walking or running movements in the hind leg are sometimes elicited from the anterior surface of the vermis, as an alternative for the inhibitory response. Since these reactions are evoked with weak currents, they also support our view that the cortex is excitable.

The internal secretion of the pancreas. F. G. Banting, C. H. Best and J. J. R. Macleod.

The hypothesis underlying our experiments was that the usual extracts of pancreas do not satisfactorily demonstrate the presence of an internal secretion acting on carbohydrate metabolism, because this is destroyed by the digestive enzymes also present in such extracts. To circumvent this difficulty we have taken advantage of the fact that the acinous, but not the insular cells become degenerated in seven to ten weeks after ligation of the ducts.

A neutral or faintly acid extract of the degenerated gland, kept at a low temperature, was therefore prepared and its effect on pancreatic diabetes investigated. Ten weeks after ligation of the pancreatic ducts the degenerated gland was removed and extracted with ice-cold Ringer's solution. This extract injected intravenously or subcutaneously invariably caused marked reduction of the percentage of sugar in the blood and the amount of sugar excreted in the urine. Extracts of liver, spleen or boiled extract of degenerated pancreas have no effect.

Further investigations have shown the following: *a*, incubation of the extract, in alkaline reaction, for 2 hours, with pancreatic juice removes its effect; *b*, glucose given intravenously or per os is retained by diabetic animals if adequate doses of the extract are also administered; *c*, the clinical condition of the animal is improved by the extract; *d*, hemoglobin estimations before and after administration of the extract are identical; *e*, neutral extract kept in cold storage retains its potency for at least seven days; *f*, subcutaneous injections have a less rapid but more prolonged effect. Rectal injections are not effective.

The experiments have been repeated on ten animals several of which were under observation for over 2 weeks.

The cardio-accelerator agent produced by hepatic stimulation. W. B. CANNON and F. R. GRIFFITH.

The accelerator effect on the denervated heart of stimulating the hepatic nerves, recently described by Cannon and Uridil, is not due to failure of the liver (during stimulation) to protect the heart from an accelerator and pressor substance absorbed from the intestine. The effect can be produced by reinjecting into the inferior cava blood drawn from the hepatic veins during stimulation. As a rule the effect of stimulation is most marked during digestion of meat. Digestion of fat and carbohydrate are without influence. Intraintestinal injections of mixed amino acids occasionally render hepatic stimulation more effective, but the results are not constant. Tyrosine may and tyramine regularly does accelerate the heart, but neither makes the nerve stimulation more effective. Neither glucose, nor urea, nor catalase causes the heart to beat faster. Fresh liver extracts in Ringer's solution accelerate the pulse, but so do fresh extracts of other organs, e.g., the salivary glands. It seems probable that a substance of special and unknown nature is discharged into the blood stream when the hepatic nerves are excited.

Latent period in reciprocal action of antagonistic muscles. J. M. D. OLMSTED and W. P. WARNER.

In decerebrate cats all the branches of the femoral nerve except that to the vastus crureus muscle, and all of the hamstring branches of the sciatic except the one to the semitendinosus were cut on the right side. Both right and left sciatic nerves were cut near the knee, and enclosed in Sherrington electrodes for centripetal stimulation. The right femur was held rigid by a pin in the lower condyle. The right leg was amputated halfway between ankle and knee and the stump attached to levers which thus recorded movements of the vastus crureus muscle. The semitendinosus was freed from its insertion and so isolated that the lever connected with it was unaffected by movements of the vastus crureus.

Upon stimulating the ipsolateral sciatic nerve a flexion reflex was elicited. When the movements of the two muscles were recorded simultaneously on a drum rotating 27.5 cm. per second, it was found that the latent period of the relaxing vastus crureus was on an average 0.014 second longer than that of the contracting semitendinosus. Likewise upon stimulating the contralateral sciatic nerve a crossed extension reflex was elicited. It was found in this case that the latent period of the relaxing semitendinosus was on an average 0.019 second longer than that of the contracting vastus crureus. Similarly if the crossed extension reflex was elicited and then the ipsolateral sciatic nerve stimulated inhibiting this reflex, the latent period of the relaxing vastus crureus was found to be 0.013 second longer than that of the contracting semitendinosus.

In the reciprocal action of antagonistic muscles, therefore, the inhibited or relaxing muscle appears to have a longer latent period than

the excited or contracting muscle. This may be due to the impulses to the former muscle having to pass through a greater number of synapses than the impulses to the latter muscle.

Physiological entities in inheritance and evolution. ERNEST L. SCOTT.

Many physiological phenomena which are variable in the lower forms become more and more constant as we ascend the scale. This implies a mechanism of control which is absent or poorly developed in the lower forms and which becomes more and more perfected as we ascend the scale.

An examination of the frequency curves for blood sugar for several forms ranging from dog-fish to man indicates that the sugar controlling mechanism is one of this group. In the more generalized types we find a curve of the low, open type, while in the more specialized forms the range of variation is shortened and a much greater proportion of the total number falls in the region of the maximum.

Again, maximum occurrence apparently falls at a greater sugar concentration in the forms in which the energy output is greatest. Thus among the rodents; the maximum frequency in a series of rabbits fell at 107; guinea pigs at 118; rats at 138 and mice at 245 mgm. per 100 cc. This is apparently in harmony with the energy output on the basis of the relation between body temperature, weight and surface. A mechanism has therefore been developed and passed on through inheritance which controls the amount of sugar in the blood and the amount of its variation about a certain norm in a given species.

In studying the curves, it is seen that some appear to be symmetrical, the distribution following the laws of chance while in others there is a distinct skew. This skew, in our opinion, represents the development of a delicate secondary mechanism permitting the animal to meet emergencies requiring sudden changes in the amount of sugar required for energy release. It is especially well developed in the cat, less so in the rabbit and is hardly apparent in the dog.

This work is confessedly incomplete, but enough data have been accumulated to indicate some of the possibilities of this method of study, and it would seem desirable not only to carry it further in the field of blood sugar but to extend it to other phases of physiology.

A study of alimentary glycemia curves in rabbits. ERNEST L. SCOTT and T. H. FORD.

Rabbits were subjected to inanition for a period of 24 hours and then fed 1, 2 or 4 grams of sugar per kilogram of body weight. Samples of blood were taken before feeding and at intervals of 30 minutes, 1, 2, and 3 hours after feeding. Controls were run in which no sugar was fed. Composite curves for each series were constructed. From them it was found that the blood sugar for the controls was almost constant throughout the period of observation. When one gram of sugar was fed, the blood sugar rose to a maximum of 175 mgm. of sugar per 100 cc. of blood at 30 minutes and had returned to the initial value at three hours

although it was still a little high at two hours. With 2 grams, a maximum of about 190 mgm. occurred at about 45 minutes and the three hour sample was still 8 per cent above the initial value. After feeding 4 grams, the maximum was 235 mgm. per 100 cc. of blood and occurred 1 hour after feeding. The three hour sample was about 20 per cent above the initial value.

It was further found that it was impossible to compare individual curves. In one case the curve for 1 gram of sugar could almost be superimposed on that for 4 grams. When however composite curves for a considerable number of observations, either on a single animal or a group were compared it was found that the results were fairly constant for a given set of conditions.

Effect of prostatectomy on integration of muscular movements of the white rat. D. I. MACHT and J. L. ULRICH.

An attempt to study the effect of prostatectomy on muscular coördination and efficiency was made by means of the so-called rope problem. White albino rats are trained to walk across a room over a tightly stretched rope starting from a platform at one end and ending at another platform on which food is placed at the other end of the room. This problem or method of study is an excellent one for the development and training of their coördination. The animals at first cannot cross the rope at all and slip off it repeatedly, hanging by the front legs. After repeated trials, however, they learn to coördinate their movements and eventually can run over the entire length of the rope rapidly and without swaying or slipping off. After such a prolonged trial one notices a marked improvement in the tonus and strength of the entire musculature of the animals. In the present investigation the effect of prostatectomy was studied on the coördination of muscular movements. Two sets of experiments were performed. In the one group of rats, the animals were trained on the rope until they mastered the problem perfectly. They were then prostatectomized and allowed to recover. After recovery the animals were quickly retrained and it was found that there was no evident change in the integration of muscular movements produced by the extirpation of the prostate glands. In the second set of experiments, another group of rats was prostatectomized after a few trials on the rope before they had completely mastered the problem. The animals were allowed to rest and after recovery from the operation training on the rope was again begun. It was soon noticed that this group of rats learned to run across the rope very poorly and indeed after even a much longer period of training than the first group of rats this second group of animals could not run over the rope in perfect fashion. The progression of the animals was much slower and more difficult. The muscles showed frequent tremblings and especially the muscles of the hind legs showed marked weakness. This was not exhibited by control rats in which laparotomy was performed but in which the prostates were not excised. In this second group of animals, a marked improvement in muscular efficiency was manifested after feeding of dried prostate and certain other glands.

The relation of parathyroid tetany to intestinal bacteria. LESTER R. DRAGSTEDT.

There is considerable evidence that certain types of tetany are associated with gastro-intestinal disturbances. Recent work on the nature of parathyroid tetany indicates that the symptoms may be due to an intoxication. An investigation of the nature of the toxemia present in intestinal obstruction has convinced the author that the major part of the toxic substances in the normal alimentary tract is produced by the action of putrefactive or proteolytic bacteria on proteins or their split products and belong to a group usually classified as ptomaines. The type of intestinal bacteria may be almost quantitatively cont rolled by the diet. Dependent upon the degree to which an aciduric or fermentative flora can be maintained the toxemia incident to obstruction can be prevented.

An aciduric intestinal flora was produced in six young dogs (2 to 6 months old) by a diet of bread and milk and milk ad libitum plus a daily ration of 25 grams of lactose. Parathyroidectomy was then done. The animals lived ten days (average) and at no time gave any evidence of tetany. They were apparently in good condition until the day of death when there was a sudden and progressive weakness. There was no marked cachexia and the animals did not die in tetany.

In a series of ten adult dogs an aciduric intestinal flora was secured by a diet consisting of boiled rice, beef heart, and lactose. The lactose was given in solution by stomach tube. Parathyroidectomy was then done and the feedings continued. Corresponding to the degree to which an aciduric flora could be maintained in the intestine the symptoms of tetany were prevented. The length of life of these parathyroidectomized animals was markedly prolonged (survived 3 to 4 weeks) and in each case death was due to pneumonia following distemper. It is probable that parathyroidectomized dogs can be kept for long periods of time perhaps indefinitely if an aciduric flora be maintained and intercurrent infections prevented.

The experiments indicate a direct relation between parathyroid tetany and intestinal intoxication and lend support to the view that the function of the parathyroid glands is in part the removal or neutralization of toxic substances in the blood. There is evidence of another and perhaps more important function indicated by the fact that the animals died even when protected from tetany. The latter symptom may be but a part of a profound metabolic derangement which will result in death even if tetany be prevented.

The influence of a beri-beri diet upon the metabolic rate of the white rat. ADDISON GULICK.

A white rat which had been kept upon a diet deficient in the water-soluble vitamines was compared as to CO_2 consumption with a brother from the same litter, which had received food adequate in kind but only in such quantities as were eaten day by day by the one on defective diet. Both brothers were stunted, holding a constant weight during

forty-five days in which a well-fed brother trebled weight. Both brothers showed basal gas exchanges, roughly one-third that of the well-fed brother. This amounts to a deficiency of 30 per cent, if the basal rate ought to vary as the two-thirds power of the body weight, and of 45 per cent if it ought to vary as the square of the body length. The deficient calorie intake must be chiefly responsible for the lowered metabolism, since there is but little difference between the two animals whose vitamine intake was different and calorie intake similar.

Pulse rate and blood pressure responses of men to passive postural changes. II. Under low oxygen. MAX M. ELLIS.

Fifteen men from the Air Service and the Medical Corps of the U. S. Army were used as subjects in these tests. The changes in position were made by means of a tilting table and with little or no muscular effort on the part of the subject. The pulse rate and blood pressures taken after ten minutes reclining constituted the normals on which the comparisons were based. Low oxygen conditions were obtained in two ways, by rebreathing and in the low pressure chamber.

The data collected showed:

1, The responses under low oxygen were of the same type as those made by the subject at sea level, until the lack of oxygen became so severe that collapse was imminent.

2, The actual amount of change in pulse rate and blood pressure varied slightly from the sea level responses until 10,000 feet equivalent altitude was exceeded.

3, Above 10,000 feet equivalent altitude the responses were more profound than those at sea level, the differences in pulse rate being the most noticeable. These responses increased as the equivalent altitude was increased.

4, Just before collapse from low oxygen the passive changes in position failed to bring about large changes in pulse and blood pressure, the tendency being for all three, i.e., pulse, systolic and diastolic pressures, to fall when the subject was tilted.

A further study of the effect of total removal of the liver. FRANK C. MANN and THOMAS BYRD MAGATH.

A large series of experiments on the effect of total removal of the liver has corroborated and enlarged on the observations we reported at the meeting last year. Total removal of the liver is always followed by a decrease in blood sugar, and a definite and characteristic group of symptoms develop related to this diminution in blood sugar. The intravenous administration of glucose after these symptoms have developed, immediately restores the animal to normal. If the blood sugar is maintained at or above the normal level, the characteristic symptoms never develop.

This vital function of the liver, of maintaining the blood sugar at a level compatible with life, has been shown by another series of experiments. The portal vein and abdominal end of the vena cava of the

liver of a normal well fed dog was connected with paraffined cannulas to the carotid artery and external jugular vein of a dog moribund after removal of the liver. The hepatic artery, common bile duct and thoracic vena cava of the transplanted liver were ligated. The blood of the moribund hepatectomized dog was allowed to circulate through the liver of the normal dog. In from fifteen to thirty minutes the blood sugar was restored approximately to its normal level and the animal appeared normal. Removal of the transplanted liver caused an immediate decrease in blood sugar and the development of the characteristic symptoms. The glycogen content of the transplanted liver decreased. We have not thus far been able to raise blood sugar in the hepatectomized animal by transplanting the approximately glycogen-free liver of a fasting animal.

The production of chronic liver insufficiency. FRANK C. MANN and THOMAS BYRD MAGATH.

The production experimentally of a chronic functional insufficiency of the liver has been difficult because of the enormous regenerative power of the organ. It is possible to remove all the liver of the dog except the two lower right lobes (approximately 70 per cent) without serious damage to the portal and vena cava circulation. The animals do not seem to suffer from such loss. The reason for this may readily be ascertained by examination a few months after operation, when the two remaining lobes will be enlarged and the amount of tissue practically the same as before operation. Hepatic insufficiency cannot be produced by this method.

If the liver is removed functionally, by ligation of the various radicles of the bile ducts draining the lobes, the same result occurs. The lobe or lobes, whose duct is left patent, enlarges and liver insufficiency is not produced. If an Eck fistula has been made some time before the removal of liver tissue, regeneration does not occur, or not to the extent to which it occurs with the portal circulation intact. In animals with an Eck fistula, therefore, it is possible to remove all the liver except the two lower right lobes and produce a definite chronic liver insufficiency.

Animals thus operated on recover from the immediate effects of the operation and for the first weeks little change is noted. Then they gradually lose weight, strength, and appetite and become emaciated. Jaundice does not occur. The only positive finding is that shortly after operation uric acid appears in the blood, and as the condition becomes progressively worse, increases in amount. In some animals the general condition improves and uric acid disappears from the blood. In these cases a certain amount of regeneration of the remaining part of the liver tissue is noted at necropsy.

From the foregoing it would seem that if a functional test is to be devised for the liver, it could be most satisfactorily developed in connection with its possible function as regards purin metabolism.

Comparison of methods of ablation of the liver. FRANK C. MANN and THOMAS BYRD MAGATH.

In order to compare the results obtained by our own method of total ablation of the liver, with the results obtained by previous investigators who removed the liver by other methods, we investigated, experimentally, their methods.

Except in instances in which the investigator had used a species of animal having the circle of Jacobson, the organ was removed by one of two methods. Some investigators made an Eck fistula and later removed the liver, lobe by lobe. Owing to the intimate relation of the vena cava and the liver, it is impossible to remove all the liver in this manner without injuring the vena cava. When we employed this method it was necessary to leave a minimum of 10 per cent of liver tissue, in order to leave the vena cava intact. If the remaining liver tissue is removed, the vena cava will be injured, producing circulatory disturbances, while if some of the tissue is left without adequate blood supply, it undergoes autolysis, and produces toxemia. Our results show that an animal from which the liver has been thus removed, rarely develops the typical symptoms of removal of the liver, because of the foregoing complicating factors.

The second method consists in removing the liver functionally by circulatory exclusion. An Eck fistula is made and later the arterial blood supply to the organ is occluded. This leaves the entire liver in the peritoneal cavity without blood supply. This method has two serious objections: *a*, it leaves an enormous amount of liver tissue without blood supply in the peritoneal cavity; autolysis and subsequent absorption of toxins is a definite complicating factor, and *b*, variations in the respiratory movements may force blood back through the vena cava into the hepatic veins and wash out material from the liver. Our experiments show that if the liver is removed functionally, the same decrease in blood sugar will occur that takes place when the organ is removed surgically. When the organ is left in place without its blood supply the animal usually develops symptoms at a blood sugar level higher than when it is completely removed. While the injection of glucose restores the animal with a functionally removed liver the first time it becomes moribund, a second injection rarely proves of any value and the animal dies within a very few hours of exclusion.

Our data tend to show that exclusion of the liver will assist in ascertaining a few facts concerning its physiology, but it is only possible to obtain clean cut facts regarding the results, when complete removal is accomplished.

The effect of total removal of the liver in lower vertebrates. THOMAS BYRD MAGATH and FRANK C. MANN.

Certain species of fresh water fishes and turtles were studied following the total removal of the liver. In the fishes used, namely the gar pike (*Lepisosteus platostomus* and *Lepisosteus osseus*), and the bowfin (*Amia calva*), the pancreas is distributed throughout the substance of

the liver so that when the liver is removed most of the pancreas was also removed. The turtle used for experimentation was the common soft shell (*Amyda spinifera*). Both the fish and turtles lived for several hours after the operation and in each case a fall in blood sugar was noted. In the case of the turtle a rise in blood uric acid was noted.

A study of fibrinogen following removal of the liver. CARL S. WILLIAMSON, FRANK J. HECK and FRANK C. MANN.

The fact that the blood of animals following hepatectomy undergoes changes in coagulability has led to the investigation of the fibrinogen following removal of the liver. Our method of investigation consisted in totally removing the liver, and at various periods after operation taking from the animal a definite amount of blood, whipping it until all fibrin was removed, and then returning it to the animal. The clot was washed carefully and the fibrinogen estimated in terms of nitrogen by the Kjeldahl method. Suitable controls of fibrinogen regeneration in normal animals, and animals with Eck fistulas before and after ligation of the arterial blood supply to the liver, were made.

The results of a large number of experiments are difficult to correlate, but it would seem that the liver is not essential for the immediate regeneration of fibrinogen.

The hepatic factor in chloroform and phosphorus poisoning. CARL S. WILLIAMSON and FRANK C. MANN

The action of some poisons in producing a definite and characteristic pathologic condition in the liver has been the basis for many studies that aimed to determine points with regard to the physiology of the organ. While the liver is markedly affected by the action of these poisons, other tissues are not unaffected by them. Our experiments on total extirpation of the liver from the dog have shown that two phenomena invariably occur with the removal of the hepatic function. The sugar decreases in the blood and uric acid appears. With this fact as a criterion, we have attempted to determine the hepatic factor in the action of chloroform and phosphorus. The method consisted of making estimations of blood sugar and uric acid before and after the administration of the drug. The results show that while blood sugar decreases somewhat and uric acid appears in small amounts, significant changes in these two constituents of the blood only occur as a terminal reaction preceding death, and that while the function of the liver is profoundly affected by chloroform and phosphorus, other factors complicate the result.

Conclusions with regard to the function of the liver, based on studies of the effect of chloroform and phosphorus poisoning must be very cautiously drawn.

The effect of some salts on the experimental amoebocyte tissue near the isoelectric point and after addition of acid and alkali. LEO LOEB and K. C. BLANCHARD.

Our results indicate that near the isoelectric point amoebocytes show the least degree of resistance to injurious influences of certain salt solutions, and that a slight addition of acid and to some extent of alkali may protect the cells not only in isotonic, but in certain cases even in hypotonic and hypertonic solutions.

Acid in combination with certain salts apparently decreases the permeability of the cells and increases their consistency. Accordingly acid has the tendency to increase the average sharpness of the pseudopods.

The blood cells of Limulus, as a result of their stereotropic response and the concomitant spreading out of their protoplasm along the surface of a solid body, undergo degenerative changes which consist essentially in processes of solution.

By making the medium into which the cells enter from the piece of amoebocyte tissue very slightly acid, the extension of the cells can be much retarded and their life and activities considerably prolonged. In alkali the cells are likewise preserved for some time, but they begin to spread out and become dissolved much earlier than in acid.

The central heat regulating mechanism. H. G. BARBOUR and E. TOLSTOI.

Investigations which have shown that dogs exposed to cold and hot baths respectively concentrate and dilute the blood, and that in the case of cold the hemoconcentration cannot be evoked in the absence of the central heat regulating mechanism, have been extended to include the regulation against heat.

The best bath temperature for obtaining hemodilution in dogs appears to be 40°C.; 38°C. baths do not cause hemodilution. In eight experiments on normal dogs 40° baths decreased the blood solids percentages by an average of 0.9. The temperature change averaged plus 0.8°C.

Five dogs after section or removal of the portion of the spinal cord underlying the sixth cervical vertebral arch placed in a 40° bath yielded an average change in the percentage of blood solids of −0.2, little or no dilution being indicated. Here the temperature increase averaged plus 3.0°C. showing the loss of the power to regulate against a hot environment. These animals furthermore do not pant in 40° baths as do normal dogs.

In 42°C. baths the normal dog exhibits some blood concentration. The percentage change in blood solids in six experiments was +0.4. Severe panting appears to be responsible for this, the water being pumped off by respiration faster than it is received from the tissues. Teleologically, unless sweating is involved, very high temperatures should be protected against by poor peripheral blood flow just as are low temperatures and this would be partly accomplished by hemoconcentration.

The thermocardiogram, and the relation of its waves to the chemical events of muscle contraction. C. D. SNYDER.

The detailed temperature oscillations of a turtle's ventricle during the cardiac cycle has been satisfactorily recorded for the surviving organ with the spontaneous beat slowed to once a minute or less. The graphic record of these temperature changes in this work is called a *thermocardiogram*.

The number of oscillations in the thermocardiogram is 5 or 6. Three are temperature rises, one a marked temperature fall, the other two are either slight falls or stases in the temperature. Each has a fixed position in the cardiac cycle and may clearly be classed with one or the other of the stages of activity and recovery of skeletal muscle known as "anoxidative" and "oxidative,"—a classification of the chemical events made by Meyerhof as he has been able to determine them.

The ratio of the algebraic sum of the heats of reaction in the anoxidative stage to the sum of all the heats of reaction of both stages, as described by Meyerhof, I calculate to be as 1:8. The heat represented by the systolic rise in the thermogram, a period I take to cover the "anoxidative" stage of M., when compared with the algebraic sum of the heats of the other temperature oscillations (excepting the marked diastolic fall, reasons for which are given in the full report of this work) has a ratio of 1 to 3.6. This indicates more heat production during systole than is demanded by the known chemical events of the anoxidative stage. If one calculates the extra heat produced by an assumed thermo-elastic effect and makes use of certain data of Hill and Hartree, the excess heat is largely accounted for.

Similar ratios of the heats of chemical reaction during the "oxidative" stage of M. and the heats of the thermocardiogram during post-diastolic and presystolic phases are in the case of the chemical reactions as 1 is to 1.14, and in the case of the thermocardiogram as 1 is to 1.10. The temperature oscillations during the post-diastolic and presystolic period of the thermocardiogram therefore fit in satisfactorily with what is demanded by the chemical events of the oxidative stage, or period of recovery, ascribed to skeletal muscle. Furthermore these latter oscillations are made up of two separate and well marked temperature rises, suggesting that the oxidation of lactic acid (that may be taken as the cause of the rises) is divided into two separate stages. The fall or stasis separating these two rises suggests a period of synthesis of part of the lactic acid into dextrose; the fall or stasis following the second post-diastolic rise suggests the period of the final chemical event as determined by Meyerhof, namely, the synthesis of dextrose to glycogen.

The glomerular circulation in the frog's kidney. A. N. RICHARDS and CARL F. SCHMIDT.

Illumination of the frog's kidney by reflected light (Krogh's method) makes it possible to study the glomerular circulation with the low power of the microscope. The blood stream in the glomerular capillaries and often-times in the afferent and efferent vessels is clearly visible.

1. The number of glomeruli showing active circulation varies widely

in different preparations. Even when the greatest precautions are taken to prevent hemorrhage or other damage to the circulation, this number is not maximal. It may be increased by injection of blood, salt solution, urea, glucose, caffeine, sodium sulphate or sodium bicarbonate. It may be decreased by constrictor doses of adrenalin or pituitrin.

2. The number of capillary loops through which blood flows in a single glomerulus is similarly variable and may be increased by the dilator agencies and decreased by the constrictor agencies mentioned above.

3. The glomerular circulation often shows a peculiar intermittence. Flow through one glomerulus may stop while that in an adjacent one continues. It may be resumed with almost equal abruptness. In many cases when blood flow stops in this manner the glomerular capillaries and the afferent and efferent vessels as well become empty of blood. This intermittence is not synchronous with the heart beats: it is apparently of peripheral origin.

Observations upon the composition of glomerular urine. JOSEPH T. WEARN.

The kidney of the frog was exposed in the manner described by Richards, then by means of a capillary pipette the capsule of Bowman was pierced and glomerular urine collected in quantities sufficient for certain tests.

Adopting the suggestion of Dr. A. N. Richards, the glomerular urine was transferred into small capillary tubes, the reagents added and the tests were then read microscopically.

It was found that any injury to the glomerular capillaries by the pipette caused a cessation of the circulation in that glomerulus. When the capsule was pierced without injury there was a temporary cessation of blood flow in the glomerular capillaries in many instances but it was soon reëstablished and continued so long as the general circulation remained good. In one instance it was observed to flow for 18 hours.

Using the potassium ferrocyanide and acetic acid test, the glomerular urine from 11 frogs was found to contain no protein. The bladder urine also showed no protein.

By McLean's method the bloods from 9 frogs were found to show no sugar. The glomerular urine from 2 of these frogs showed no sugar. Small injections of glucose were then given subcutaneously and the glomerular urine from 7 frogs so treated was found to show sugar by Benedict's test. The bladder urine collected during the same period showed no sugar. Larger injections of glucose were then given and sugar was found in both the glomerular and bladder urine.

Using the silver nitrate and nitric acid test, chloride was found to be present in the glomerular urine from 4 frogs and absent in the bladder urine collected during the same period. In two other instances chloride was found to be present in both glomerular and bladder urine.

Similar studies are being made upon other substances.

These results are offered as proof of reabsorption in the tubules and as strong evidence in favor of glomerular filtration.

Experimental proof of vagus apnea. DON R. JOSEPH.

There has been much discussion and also experimental effort expended on the question of whether the respiratory center is guided by the gaseous content of the blood or by afferent nervous impulses coming particularly over the vagus nerves from the lungs. In recent years so much emphasis has been laid upon the importance of CO_2 in this respect that the existence of a nervous factor has been questioned and even denied in spite of much evidence that pointed in the opposite direction. The uncertainty has arisen apparently from the fact that in experiments devised to demonstrate a nervous control over the respiratory center the possibility of a simultaneous change in gaseous content of the blood was not ruled out.

To supply this evidence we have used the following method: Under ether anesthesia, after tracheotomy, the chest and abdomen of a dog are laid wide open under artificial respiration; a portion of the diaphragm with intact blood and nerve supply is connected to a muscle lever; finally one lobe of one lung is plugged off by means of a small tube introduced through an opening in the trachea just below the tracheal cannula. Artificial respiration is maintained at such a level that the diaphragm shows rhythmic contractions of moderate intensity. With this preparation the following observations have been made:

1. Inflation of the plugged lobe produced by blowing through the rubber tubing and while continuing uninterruptedly the artificial respiration in all the rest of the lung gives prompt relaxation (inhibition) of the diaphragm which may persist for many seconds if the inflation is sustained. We believe it cannot be said that during this inflation the gaseous content of the blood has been altered since ventilation of all the remainder of the lung continues unchanged throughout the period of inflation of the single lobe.

2. This inhibition cannot be produced after both vagi have been cut.

3. If artificial respiration be discontinued and the whole lung permitted to collapse, the diaphragm, as is well known, goes into a sustained tetanus. Distention of the plugged lobe will now cause a prompt inhibition of this tetanus, and its deflation be followed by an equally prompt return of the tetanus. In fact a series of such relaxations of the tetanus of the diaphragm can be produced by alternately blowing and sucking through the tube leading to the plugged lobe. The diaphragmatic rhythm will follow exactly that of the lobe.

4. Cutting the vagi destroys this synchronism.

5. We believe this observation explains why the spontaneous respiratory movements so often correspond to the rate of artificial respiration.

6. Evidence already at hand warrants the statement that these afferent nerve impulses coming from the lungs spread in the central nervous system beyond the limits of the respiratory center, affecting the cardio-inhibitory and perhaps other centers. This furnishes added proof of the existence of the nerve impulses in question. The investigation is still in progress.

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PURPOSES

The main purpose of the Physiological Reviews is to furnish a means whereby those interested in the physiological sciences may keep in touch with contemporary research. The literature, as every worker knows, is so extensive and scattered that even the specialist may fail to maintain contact with the advance along different lines of his subject. The obvious method of meeting such a situation is to provide articles from time to time in which the more recent literature is compared and summarized. The abstract journals render valuable assistance by condensing and classifying the literature of individual papers, but their function does not extend to a comparative analysis of results and methods. Publications such as the Ergebnisse der Physiologie, the Harvey Lectures, etc., that attempt this latter task, have been so helpful as to encourage the belief that a further enlargement of such agencies will be welcomed by all workers. It is proposed, therefore, to establish a journal in which there will be published a series of short but comprehensive articles dealing with the recent literature in Physiology, using this term in a broad sense to include Bio-chemistry, Bio-physics, Experimental Pharmacology and Experimental Pathology.

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